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DEVELOPMENT OF PEPTIDE COUPLED TRIAZOLE MOLECULE AS POTENT ANTICANCER THERAPEUTIC AGENT FOR THE TREATMENT OF PROSTATE CANCER

Chemical Science

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ABSTRACT

Prostate cancer is thought to have claimed the lives of nearly 42,000 men worldwide in 2024 alone, despite significant improvements in diagnosis and treatment. Since androgens contribute significantly to the development of prostate tumours, the androgen receptor (AR) is the main target for PC treatment. There are currently no authorized therapeutic targets for androgen receptor castrate resistance prostate cancer (CRPC), an aggressive kind of prostate cancer. Four peptides' cytotoxic potential in AR and AR+ cell lines was investigated in this work. A biological evaluation showed that the triazole core increased the overall activity of AR-antagonists. Indeed, using both androgen-dependent and androgen-independent pathways, the representative drug SR1d demonstrated encouraging invitro antitumor efficacy against three distinct 22Rv1, LNCaP, and PC3 PC cell lines, suppressing tumour development by 60%. Additionally, in a xenograft mice model, SR1d inhibited the growth of prostate tumours. In the therapy of CRPC tumours, this shows great promise for advanced preclinical research.

KEYWORDS

Prostate Cancer (PCa); Androgen Receptor; AR-Antagonist; Anti-prostate Cancer Agents; Antiproliferative; CuAAC/click Chemistry,

INTRODUCTION

Cancer is a chronic illness brought on by the body's cells proliferating too much and becoming uncontrollably [1]. With a high incidence and fatality rate, prostate cancer (PCa) is the most prevalent malignant tumour in males among all cancer types [2]. The underlying aetiology of prostate cancer is still unclear, and the illness is complex and heterogeneous, acting differently in various individuals. According to a number of studies, androgen and the androgen receptor (AR) are thought to be important factors in the development of PCa. Currently the primary therapeutic techniques used for treatment of PCa are, surgery, radio-therapy, chemotherapy and immunotherapy. Even though solid tumours can be swiftly removed with surgery, this may be a useful therapeutic approach for early or middle-stage tumours. For the majority of patients with advanced tumours, this type of treatment is not the ideal option because it frequently results in trauma, bleeding, infection, decreased immunity, and other dangers [4]. Patients who have not benefited from surgical treatment are frequently treated with radiation therapy, which is a costly and drawn-out procedure. A number of problems are frequently the outcome of this treatment [5]. Chemotherapy is the process of attacking cancer cells by introducing chemicals into the body. The two therapeutic agents that are clinically utilised to treat prostate cancer are nonsteroidal antiandrogens like flutamide and bicalutamide and steroidal antiandrogens like cyproterone acetate. Due to their partial agonistic action and overlapping effects with other hormonal systems, steroidal antiandrogens can cause a number of difficulties, including erectile dysfunction, gynaecomastia, and serious cardiovascular issues [6–8]. In comparison to steroidal antiandrogens, nonsteroidal antiandrogens are preferred due to their better oral absorption and fewer adverse effects. However, patients who have been taking nonsteroidal antiandrogens for a number of months have been known to experience antiandrogen withdrawal syndrome [9, 10]. In addition to killing tumour cells and healthy cells equally, long-term use of these medications has clear side effects include mutation of the AR and increases susceptibility to drug resistance with a very high chance of recurrence [11]. Prostate adenocarcinoma continues to have a high incidence and accounts for 20% of cancer-related fatalities in men, despite improvements in diagnostic and therapeutic treatment techniques [12]. Prostate cancer is driven by the androgen receptor (AR), according to a number of studies [13, 14]. Since the majority of PCa cells initially rely on androgens to survive, lowering androgen levels is the first line of treatment for PCa [15, 16]. Effective new Androgen receptor down-regulating drugs (ARDAs) are desperately needed for the prevention and treatment of advanced metastatic PC, as evidenced by the disease's dismal clinical prognosis [17–22].

Peptide conjugates, a new class of anticancer medications that can specifically target cancer cells with minimal damage to healthy organs, may improve future cancer therapy choices [23–25]. Peptides that specifically attach to cancer cell surface receptors may enhance tumour therapy. By obstructing oncogenic signalling pathways, these peptides prevent cancer cells from dying, regulate their activity, and promote the proliferation of tumour cells [26].

Solid-phase Peptide Synthesis

The peptides SR1a-d used in this study were synthesized using solidphase peptide synthesis [27,28]. The general synthetic scheme for the peptides is illustrated in figure 1. The synthesis of peptides SR1a to SR1d was carried out in a frit equipped, acid and base resistant syringe on rink amide resign. For loading the first amino acid, the resin was pre-swollen in dimethylformamide (20 min). The Fmoc group removed by treating the resin with 1.5 mL of 20% pipiridine in dimethylformamide for two times and resin was washed with dimethylformamide. Then added to a solution of the amino acid derivative, HATU and HOBt (3 eq. each) were dissolved in 2.5 ml of DMF in a separate test tube and for this DIPEA (5 eq.) was added to this solution and allowed to react for 10 min at rt. The resulting suspension was added to the resin and the reaction mixture was left shaking for 5 h at rt.

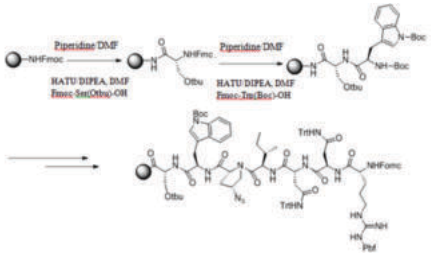


Figure 1: Flow Chart of Solid-based Peptide Synthesis Steps

After obtaining a homogeneous solution, six equivalents of diisopropylethylamine (DIEA) was added to the solution. The resulting mixture was then added to the resin and gently Shaked using shaker for 5 hr. After completion of the coupling which was confirmed by ninhydrin test, the resin was then washed and solvent was removed. The resin was then treated 2 times for 30 s each time with 1.5 mL of 20% Piperidine in dimethylformamide to remove the Fmoc group and washed thoroughly with dimethylformamide solvent. Subsequent amino acids were added to the peptide sequence by repeating the procedures described above.

To couple the each amino acid moiety on to rink amide resin, first corresponding amino acid was placed in a test tube containing the HATU coupling agent and activator HOBt (three equivalents), they were completely dissolved using dimethylformamide.

Construction of Triazole and Amide Groups at the g Position of Proline

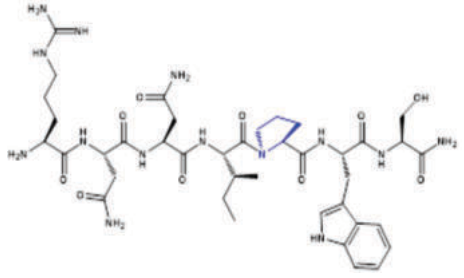
Reduction of Azide Group to the Amine Functionality on Solid-phase

Reduction of azide group to the amine functionality on solid-phase peptide was carried out using reported procedure [29]. For this the resin bound peptide systems were exposed to PPh_3, in DMF: water (25% v/v) at room temperature for 24hr. the resin was washed with DMF and acylation reaction was carried out with p-aminobenzoic acid using regular peptide coupling procedure. After the acylation was completed the resin was washed with DMF followed by dichloromethane and dried. The peptide was cleaved from the resin with trifluoroacetic acid and purified by HPLC.

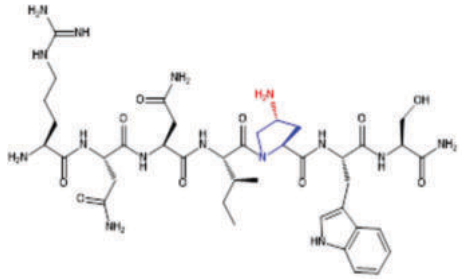
The [3+2] Cycloaddition Reaction of Peptidyl Azidoproline

The copper-catalyzed azide-alkyne cycloaddition (CuAAC) "click" reaction was chosen for its selectivity in azide and alkyne conjugation to further modification of peptide SR1a. Click chemistry has been carried out with p-aminophenylacetylene using a previously reported on-resin methodology [30]. The resin was suspended in a mixture of acetonitrile, water, DIEA, and pyridine (4:4:2:1). The alkyne compound was added followed by a catalytic amount of CuI. After shaking overnight, the resin was washed with DMF followed by dichloromethane and dried. Finally the peptide was cleaved from the resin with trifluoroacetic acid and purified by HPLC.

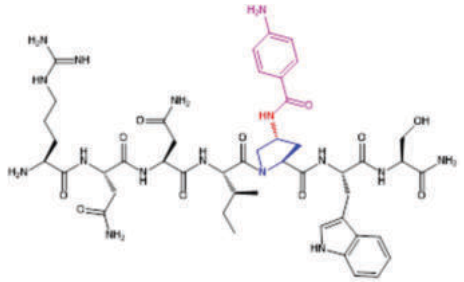
The innovative peptide (NH_2-Arg-Asn-Asn-Ile-Pro-Trp-Ser-CO-NH_2) SR1a and its derivatives (Figure 2) were synthesized using solid phase methods. Replacing the proline residue with modified azidoproline residue gave peptide SR1b. Reduction of azide group on resign using PPh_3 DMF: H_2O followed by acylation with p- aminobezoicacid gave compound SR1c. Compounds SR1d was obtained by click reaction with p-aminophenylacetylene.



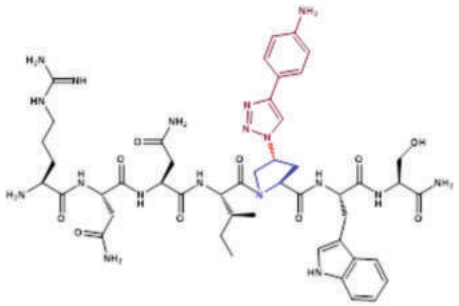
SR1a



SR1b



SR1c



SR1d

Figure 2: Structure of SR1a, SR1b, SR1c and SR1d Compounds

Cleavage of Peptides from the Resin

Peptides attached with resin were stirred with cocktail: TFA: H_2O: triisopropylsilane = 95:2.5:2.5 for 3 h. Crude peptide was filtered and washed with methanol solvent. The solvent was evaporated using nitrogen gas (N_2). Diethyl ether was then added to the crude to precipitate the peptide. The peptide was separated by centrifugation. This step of washing with ether was repeated five times and finally the remaining sediment was dissolved in water and acetonitrile and placed in a freeze dryer. The peptides produced were characterized using high-performance liquid chromatography (gradient from 0% to 100% MeCN for 20 min, C18 column) and a purity of more than 95% was collected, dried using lyophilizer and the structural integrity of the peptides was characterized by mass spectroscopy.

Biology

Cell Toxicity Study

Five human cancer cell lines, MCF-7, HCT116, HeLa HepG2 and LNCaP were used to determine the anti cancer activity of the 4 peptides. Normal human cell line HaCaT was also used for comparison. Cell toxicity experiments were carried out in accordance with the previously reported method [31, 32]. Cell viability was examined by employing the MTT technique which its principle is on the basis of the transformation of 3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye to formazan crystal by succinate dehydrogenase enzyme of mitochondria in the alive cells.

The cells were bred into 96-well plates at a concentration of 10^4 cells/well and incubated for 24 h. The cells were exposed to different serial dilution (0, 2.5; 5.0; 10.0; 20.0; 40.0, and 80.0 µM) concentrations of the peptides for 48 h. The supernatant solution was removed and MTT reagent (10 µL, 5 mg/mL in PBS) was added to each well and the microplate was kept at 37°C for 4 h. The medium solution containing MTT was discarded and DMSO (100 µL) was added to each well to dissolve the formazan crystals. The plates were then maintained for 20 min at 37 °C. At the end, the optical density of each well was read at 570 nm against the reference wavelength of 630 nm as the background, employing a spectrophotometer plate reader (Infinite® M200, TECAN) [33]. For breast cancer cells, ciprofloxacin as a positive cytotoxic control of the peptides was used. Data were shown as the mean of triplicate measuring of the number of living cells.

RESULTS

In Vitro Antitumor Activity of Peptides

For cellular assessments, a select panel of cancer cell lines was initially evaluated including MCF-7 human breast adenocarcinoma cells, HCT116 human colon adenocarcinoma cells, HeLa cervical adenocarcinoma cells, HepG2 hepatocellular carcinoma cells, LNCaP androgen-sensitive human prostate cancer cell line and HaCaT normal human epidermal keratinocytes cell line were used to study the antiproliferative activity, the growth and colony formation.

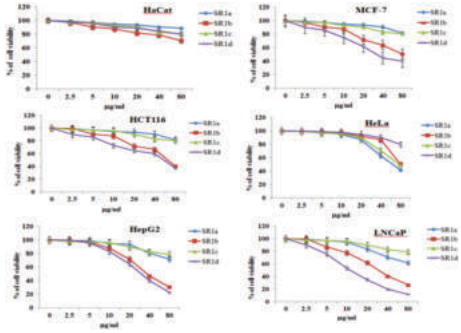


Figure 3: The effect of Peptides SR1a-SR1d for inhibition of growth

and colony formation on HaCaT, MCF-7, HCT116, HeLa, HepG2 and HL-60 cells. Data plotted are mean values ±SEM for three independent experiments.

As shown in Figure 3, the cellular antiproliferation activities of peptides SR1b and SR1d against all tested cell lines were relatively high, while those of Peptides SR1a and SR1c were 100 times lower than peptides SR1b and SR1d.

The cellular antiproliferation activities SR1b and SR1d against the growth and colony formation are considerably higher for HCT116 and HeLa cell lines but significantly high for HepG2 and LNCap cell lines. Though Peptides SR1a and SR1c were inhibit the growth and colony formation of HeLa, HepG2 and LNCap cell lines at higher concentrations (>50μM) and these peptides are not able to significantly inhibit the growth and colony formation of MCF-7 and HCT116 cell lines even at higher concentration (> 80μM). An ideal anticancer drug should be selectively toxic against cancer cells with minimal toxicity to normal cells. We have evaluated the toxicity against normal human epidermal keratinocytes HaCaT cell line. It should be noted that all the peptides SR1a-SR1d were show minimal toxicity against normal human epidermal keratinocytes HaCaT cell line.

The compounds SR1c and SR1d demonstrated significant inhibition of HeLa, HepG2, LNCaP cells proliferation and colony formation. The results of the analysis were expressed as IC_{50} and shown in Table 1. Compound SR1d exhibit cytotoxic effect on HeLa (31 μM); HepG2 (25 μM) and LNCaP (16 μM) whereas compound SR1b exhibit cytotoxic effect on HeLa (37 μM); HepG2 (32 μM) and LNCaP (40 μM) respectively. These results indicate that compound SR1d is very effective in cytotoxic effect on LNCaP cell line and 3 times more active than compound SR1b.

Table 1. Antiproliferative Activity of Tested Extracts, IC_{50}, μg/ml.

No	Peptides	MCF-7	HCT116	HeLa	HepG2	LNCaP	HaCaT
1	SR1a	>100	>100	> 50	> 50	> 50	>100
2	SR1b	> 50	≈ 50	37±0.64	32±0.43	40±1.4	>100
3	SR1c	>100	>100	34±1.2	> 50	> 50	>100
4	SR1d	> 50	≈ 50	31±0.9	25±0.4	16.3±0.3	>100

In Vitro Antitumor Activity of Compound SR1d in Prostate Cancer

LNCaP cell line is an androgen-sensitive human prostate cancer cell line. [34] Several studies suggest that prostate cancer is dependent on androgens at initial stage for growth. [35] Over the time, the cancer can progress to an androgen independent form and classified as castrate-resistant prostate cancer (CRPC). In this stage tumors can grow in the absence of androgens.[36] Since the compound SR1d was most effective for LNCaP androgen-sensitive human prostate cancer cell line, further analysis was performed with androgen-independent human prostate cancer cell lines including PC3 and DU-145 and compared with LNCaP.

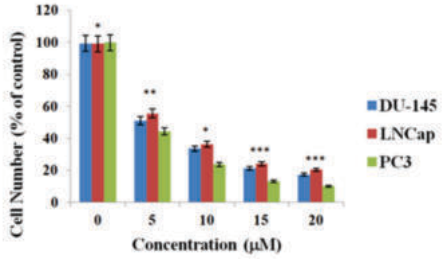


Figure 4: The effect of peptide SR1d on cell viability of different Prostate Cancer cells in a dose-dependent manner. *p < .05, **p < .01, and ***p < .001

The cytotoxic potential of SR1d was examined in PC3, LNCaP and DU-145 cells. The result showed that SR1d elicited significant cytotoxicity toward all the three cell lines (Figure 4) with the lowest EC_{50} reported in PC3 cell. cytotoxicity was more pronounced in PC3 cells with an EC_{50} of 10.3 ± 0.5 μM (Table 2).

Table 2. Inhibitory Activities of Compound SR1d Against Other PCa Cell Lines (EC_{50} values (μM))

Peptide	PC3	DU-145	LNCaP
SR1d	10.3 ± 0.5	14.2 ± 0.4	16.3 ± 0.3

In several cancer cells like leukemia, breast cancer and skin squamous AKR1C3 is reported to be involved in cell proliferation through activation of MAPK (mitogen-activated protein kinase) or PI3K (phosphatidylinositol-3 kinase pathway).[37-40] PC3 cells generally express AKR1C3 but not AR. The Significant reduction of viable cell numbers in the presence of SR1d compound indicating that AKR1C3 plays an important role in the androgen-independent PC3 cell proliferation.

Effect of Peptide SR1d on Androgen-dependent Proliferation of Prostate Cancer 22Rv1 cells

It is well established that 22Rv1 cells expressed both AKR1C3 and AR,[41] therefore we selected this cells for evaluate the effect of peptide SR1d on androgen-dependent proliferation. Initially we carry out the effect of pregnenolone, a precursor of androgens promotion on proliferation of the cells. The promotive effect of pregnenolone was found to be in a dose-dependent manner and was maximum at 20 μM, (Figure 5A) hence the cells were treated with 20μM of pregneolone in subsequent proliferation experiments.

Suppression of the pregneolone-induced proliferation with SR1d was studied and compared with AR antagonist flutamide. The result showed that proliferation of pretreated cells with SR1d significantly suppressed compare to flutamide (Figure 5B). Next we carried out the flow cytometer study to analyze the changes in the cell cycle on treatment of SR1d. It is observed that treatment of pregnenolone significantly increased S-phase cell population and pretreatment with SR1d caused decrease in both S-phase and the G0/G1-phase cell population (Figure 5C). These result indicate that peptide SR1d arrest the cell in the G0/G1-phase.

Next we tested weather the anti-proliferative effects of peptide SR1d is from suppression of androgen signaling through inhibition of AKR1C3 in the cells, is effects on cellular metabolism of 4-androstene-3,17-dione (4AD) to testosterone was examined by quantifying testosterone concentration. For this we first quantified the testosterone concentration by pretreatment with finasteride an inhibitor of steroid 5α- reductase that catalyzes the conversion of testosterone to DHT. It was found that the testosterone concentration was doubled by the pretreatment with 2μM finasteride. Then cells were cultured under 2μM finasteride pretreated conditions with 0 and 20 μM SR1d peptide. The presence of 20 μM SR1d peptide significantly reduces the testosterone production (Figure 5D). Further it was noticed that in the absence of finasteride, the production of testosterone doesn't effected significantly by the treatment of SR1d peptide. This suggested that the peptide SR1d do not affect catalytic potency of 5α reductase.

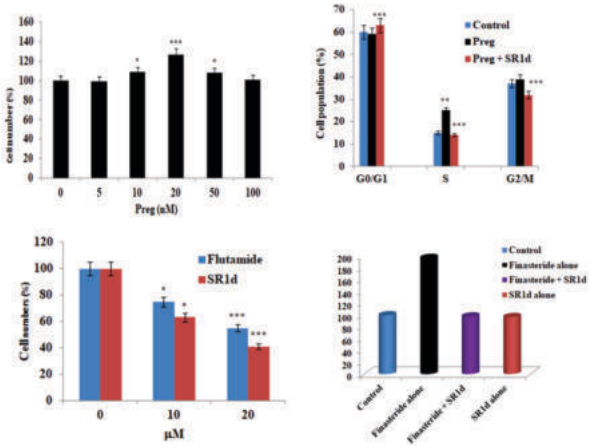


Figure 5: Suppression of androgen-dependent proliferation of prostate cancer 22Rv1 cells by SR1d.

(A) Cell proliferation by pregnenolone (Preg). The cells were cultured in the absence or presence of the indicated concentrations of Preg, and the viable cell numbers were estimated at 72 h. Data are expressed as percentages of the cell number in the Preg-free control cells. Significant difference from the control cells, ***p < 0.01, *p < 0.05.

(B) Effects of SR1d and flutamide on the Preg induced cell proliferation. The cells were pretreated for 2 h without or with the indicated concentrations of SR1d, or flutamide and then treated for 72 h with 20 nM Preg. Data are expressed as percentages of the cell numbers in the Preg-free control cells.

(C) Effects of SR1d on the cell cycle. The cells were pretreated for 2 h

without or with 10 μM 20 SR1d and then treated for 24 h with 20 nM Preg. The percentages of G0/ G1, S, and G2/M phases were analyzed using a flow cytometer.

(D) Effects of SR1d on the metabolism of 4AD into testosterone. The cells were treated for 2 h without or with 2 μM finasteride and/or AKR1C3 inhibitor (SR1d) and then cultured for 24 h in the presence of 100 nM 4AD. The concentration of testosterone in the cell extracts was analyzed by EIA and is expressed as the percentage of that in the finasteride-free control cells.

Significant difference from the control cells (***< 0.001) (**p < 0.01) and (*p < 0.05).

Effect of SR1d on Prostate Tumor Growth

To evaluate the anticancer efficiency of the SR1d in vivo we investigated the suppressive effect of this compound on prostate tumor growth in a xenograft mouse model using PC3 cells. Male muse bearing orthotropic prostate tumors were administrated a daily oral does of vehicle, and SR1d (8.5 mg/kg). The results showed that SR1d treatment significantly reduced tumor volume by 66% (1900 ± 204 vs 650 ± 73) compared to vehicle (Figure 6). Intrestingly no histopathological changes were observed in other tissues of the lung, liver, and kidney of the SR1d treated mouse. Thisresults suggest a possibility that the SR1d is useful as novel therapeutic agent for CRPC.

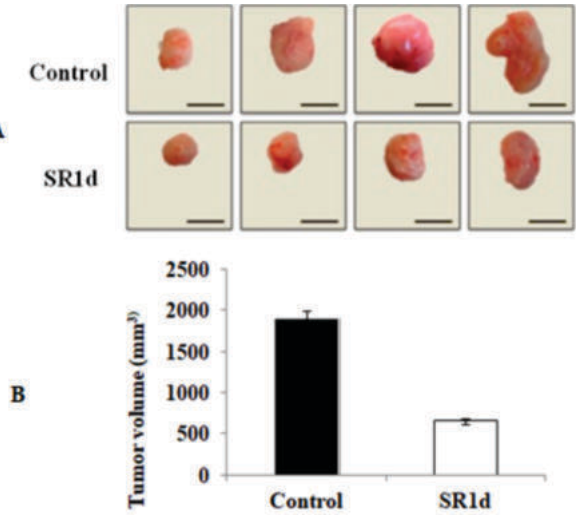


Figure 6: Effect of SR1d on tumor growth in a xenograft mouse. Male mice were inoculated with 2 x 105 PC3 cells directly to the ventral prostate. Mice were treated with either vehicle (1:1 peanut oil: water mL/kg), SR1d (8.5mg/kg) by oral gavage daily for six weeks. Necropsies were formed 24h after the last day of treatment (A) Photograph of the prostate tumors from each treatment group. (B) Mean ± SEM of tumor volume. The prostate of an animal that did not receive PC3 cell inoculation was taken as reference. Scale bar represents 10mm.

CONCLUSION

AR-prostate cancer has a poor prognosis, with the majority of patients dying within two years and no clinically approved targeted treatment options [42,43]. Thus, there is a need to discover treatments that target prostate cancer cells independently of the AR. Many synthetic and natural inhibitors for AKR1C3 have been reported. Though development of selective inhibitor for AKRIC3 is desire, since AKR1C3 is structurally similar to other AKRIC isoforms like AKR1C1, AKR1C2 and AKR1C4 having different physiological roles. In this study we have developed a novel peptide triazole as potent AKR1C3 inhibitor with high selectivity of this enzyme over other human AKRIC subfamily members. Our in vivo and in vivo studies demonstrated that SR1d inhibitors exhibit excellent antiproliferative activity against prostate cancer Pc3, DU-145, and LNCaP cells and also 22Rv1 cells through suppression of androgen signaling by dual inhibition of AKR1C3 and AR. The inhibitors SR1d suppressed the proliferation of androgen independent and androgen-low-sensitive prostate cancer cells. In line with these findings, our current research demonstrates that peptide triazole compounds may be potential therapeutics for the treatment of prostate cancer. Further studies to reveal the underlying molecular mechanism of prostate cancer inhibition are required to explore the therapeutic potential of the peptide.

Acknowledgments

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Institutional Review Board Statement: The animal study protocol was approved by the Animal Ethics Committee at the University of Mysore (protocol number 2/14 approved on 13 February 2014).

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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

REFERENCES

1. R. J. Boohaker, M. W. Lee, P. Vishnubhotla, J. M. Perez, A. R. Khaled, The use of therapeutic peptides to target and to kill cancer cells. Curr. Med. Chem. 19(22) (2012) 3794–3804. https://doi.org/10.2174/092986712801661004.
2. R. L. Siegel, A. N. Giaquinto, A. Jemal, Cancer statistics, 2024. CA Cancer J. Clin. 74(1) (2024) 12−49. https://doi.org/10.3322/caac.21820. Epub 2024 Jan 17.
3. R. K. Ross, M. C. Pike, G. A. Coetzee, J. K. Reichardt, M. C. Yu, H. Feigelson, F. Z. Stanczyk, L. N. Kolonel, B. E. Henderson, Androgen Metabolism and Prostate Cancer: Establishing a Model of Genetic Susceptibility. Cancer Res. 58(20) (1998) 4497–4504.
4. Z. Cai, Y. Yin, C. Shen, J. Wang, X. Yin, Z. Chen, Y. Zhou, B. Zhang, Comparative effectiveness of preoperative, postoperative and perioperative treatments for resectable gastric cancer: a network meta-analysis of the literature from the past 20 years. Surg. Oncol. 27(3) (2018) 563- 574. https://doi.org/10.1016/j.suronc.2018.07.011.
5. W. D. Yu, G. Sun, J. Li, J. Xu, X. Wang. Mechanisms and therapeutic potentials of cancer immunotherapy in combination with radiotherapy and/or chemotherapy. Cancer Lett. 452 (2019) 66–70. https://doi.org/10.1016/j.canlet.2019.02.048.
6. S. L. Goldenberg, N. Bruchovsky, Use of Cyproterone Acetate in Prostate Cancer. Urol. Clin. North. Am. 18 (1) (1991) 111–122. https://doi.org/10.1016/S0094-0143(21)01398-7.
7. de Voogt, H. J. The position of cyproterone acetate (CPA), a steroidal anti-androgen, in the treatment of prostate cancer. Prostate 24(S4) (1992) 91–95. https://doi.org/10.1002/pros.2990210514.
8. H. J. de Voogt, P. H. Smith, M. Pavone-Macaluso, M. de Pauw, S. Suciu, Cardiovascular Side Effects of Diethylstilbestrol, Cyproterone Acetate, Medroxyprogesterone Acetate and Estramustine Phosphate Used for the Treatment of Advanced Prostatic Cancer: Results from European Organization for Research on Treatment of Cancer Trials 30761 and 30762. J. Urol, 135(2) (1986) 303–307. https://doi.org/10.1016/S0022-5347(17)45620-5.
9. W. K. Kelly, H. I. Scher, Prostate Specific Antigen Decline after Antiandrogen Withdrawal: the Flutamide Withdrawal Syndrome J. Urol 149(3) (1993) 607–609. https://doi.org/10.1016/S0022-5347(17)36163-3.
10. H. Suzuki, K. Akakura, A. Komiya, S. Aida, S. Akimoto, J. Shimazaki, Codon 877 Mutation in the Androgen Receptor Gene in Advanced Prostate Cancer: Relation to Antiandrogen Withdrawal Syndrome. Prostate, 29(3), (1996) 153–158. https://doi.org/10.1002/1097-0045(199609)29:3<153::AIDPROS2990290303>3.0.CO;2-5.
11. C. E. Bohl, W. Gao, D. D. Miller, C. E. Bell, J. T. Dalton, Structural basis for antagonism and resistance of bicalutamide in prostate cancer. Proc. Natl. Acad. Sci. U.S.A 102(17) (2005) 6201–6206. https://doi.org/10.1073/pnas.0500381102.
12. F. Aljabery, I. Shabo, O. Gimm, S. Jahnson, H. Olsson. The expression profile of p14, p53 and p21 in tumour cells is associated with disease-specific survival and the outcome of postoperative chemotherapy treatment in muscle-invasive bladder cancer. Urol. Oncol. 36(12) (2018) 530.e7-530.e18. https://doi.org/10.1016/j.urolonc.2018.05.025.
13. R. L. Siegel, K. D. Miller, A. Jemal, Cancer statistics, 2017. CA Cancer J. Clin. 67 (3) (2017) 177–193. https://doi.org/10.3322/caac.21395.
14. C. Dai, H. Heemers, N. Sharifi, Androgen signaling in prostate cancer. Cold Spring Harb. Perspect. Med. 7(2017) a030452. https://doi.org/10.1101/cshperspect.a030452.
15. T.A. Yap, A. D. Smith, R. Ferraldeschi, B. Al-Lazikani, P. Workman, J. S. de Bono, Drug discovery in advanced prostate cancer: Translating biology into therapy. Nat. Rev. Drug Discov. 15 (2016) 699–718. https://doi.org/10.1038/nrd.2016.120.
16. M. J. Connor, T. T. Shah, G. Horan, C. L. Bevan, M. Winkler, H. U. Ahmed, Cytoreductive treatment strategies for de novo metastatic prostate cancer. Nat. Rev. Clin. Oncol. 17 (2020) 168−182. https://doi.org/10.1038/s41571-019-0284-3.
17. M. E. Taplin, S. P. Balk, Androgen receptor: A key molecule in the progression of prostate cancer to hormone independence J. Cell. Biochem. 91(3) (2004) 483–490. https://doi.org/10.1002/jcb.10653.
18. A. F. Santos, H. Huang, D. J. Tindall, The androgen receptor: a potential target for therapy of prostate cancer. Steroids 69(2) (2004) 79–85. https://doi.org/10.1016/j.steroids.2003.10.005.
19. C. D. Chen, D. S. Welsbie, C. Tran, S. H. Baek, R. Chen, R. Vessella, M. G. Rosenfeld, C. L. Sawyers, Molecular determinants of resistance to antiandrogen therapy. Nat. Med. 10 (2004) 33–39. https://doi.org/10.1038/nm972.
20. H. Suzuki, T. Ueda, T. Ichikawa, H. Ito, Androgen receptor involvement in the progression of prostate cancer. Endocr. Relat. Cancer 10 (2) (2003) 209–216.
21. J. L. Mohler, C. W. Gregory, O. H.Ford, D. Kim, C. M. Weaver, P. Petrusz, E. M. Wilson, F. S. French, The Androgen Axis in Recurrent Prostate Cancer. Clin. Cancer Res. 10(2) (2004) 440–448. https://doi.org/10.1158/1078-0432.CCR-1146-03.
22. J. A. Becker, K. D. Berg, M. A. Røder, K. Brasso, P. Iversen, Cytoreductive prostatectomy in metastatic prostate cancer: a systematic review. Scand. J. Urol. 52(1) (2018) 1−7. https://doi.org/10.1080/21681805.2017.1363816.
23. Chou K. C., Review: Structural bioinformatics and its impact to biomedical science. Curr. Med. Chem. (2004) 11: 2105–34.
24. R. Zhang, D. Q. Wei, Q. S. Du, Molecular modeling studies of peptide drug candidates against SARS. Med. Chem. 2 (3) (2006) 309–14. https://doi.org/10.2174/157340606776930736.
25. D. Q. Wei, Q. S. Du, S. Sirois, W. Z. Zhong, Progress in computational approach to drug development against SARS. Curr. Med. Chem. 13 (27) (2006) 3263–70. https://doi.org/10.2174/092986706778773077.
26. M. Karami Fath, K. Babakhaniyan, M. Zokaei, et al. Anti-cancer peptide-based therapeutic strategies in solid tumors. Cell Mol Biol Lett. 27(1) (2022) 33. https://doi.org/10.1186/s11658-022-00332-w.
27. J. Michl, B. Scharf, A. Schmidt, et al. PNC-28, a p53-derived peptide that is cytotoxic to cancer cells, blocks pancreatic cancer cell growth in vivo. Int J Cancer. 119(7) (2006)1577-1585. https://doi.org/10.1002/ijc.22029.
28. I. Coin, M. Beyermann, M. Bienert, Solid-phase peptide synthesis: from standard procedures to the synthesis of difficult sequences. Nat Protoc. 2(12) (2007) 3247-3256. https://doi.org/10.1038/nprot.2007.454.
29. N. Natalia, G. Valerie, C. Aviv, D. Saviana, A. L. Hilal B. Ashraf, Switch Peptide via Staudinger Reaction. Org. Lett., 10(22) (2008) 5243-5246. https://doi.org/10.1021/ol802268e.

30. H. N. Gopi, K. C. Tirupula, S. Baxter, S. Ajith, I. M. Chaiken, Click chemistry on azidoproline: high-affinity dual antagonist for HIV-1 envelope glycoprotein gp120. ChemMedChem 1(1)(2006) 54–57. https://doi.org/10.1002/cmdc.200500037.
31. F. Mohammadpour, S. N. Ostad, S. Aliebrahimi and Z .Daman. Anti-invasion effects of cannabinoids agonist and antagonist on human breast cancer stem cells. Iran. J. Pharm. Res. 16, (2017) 1479-86.
32. A. Rabzia, M. Khazaei, Z. Rashidi and M. R. Khazaei. Synergistic Anticancer Effect of Paclitaxel and Noscapine on Human Prostate Cancer Cell Lines. Iran. J. Pharm. Res. 16, (2017) 1432-42.
33. T. Mosmann, Rapid colorimetric assay for cellular growthand survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 65, (1983) 55-63.
34. P. Saraon, A. P. Drabovich, K. A. Jarvi, E. P. Diamandis, Mechanisms of Androgen-Independent Prostate Cancer. EJIFCC 25, (2014), 42–54. https://doi.org/10.1016/j.eururo.2012.02.054.
35. Y. Huang, X. Jiang, Liang, X. G. Jiang, Molecular and cellular mechanisms of castration resistant prostate cancer (Review). Oncol. Lett. 15, (2018), 6063–6076. https://doi.org/10.3892/ol.2018.8123.
36. A. O. Sartor, Progression of metastatic castrate-resistant prostate cancer: Impact of therapeutic intervention in the post-docetaxel space. J. Hematol. Oncol. 4, (2011), 18. https://doi.org/10.1186/1756-8722-4-18.
37. J. C. Desmond, J. C. Mountford, M. T. Drayson, E. A.Walker, M. Hewison, J. P. Ride, Q. T. Luong, R. E. Hayden, E. F.Vanin, C. M. Bunce, The aldo-keto reductase AKR1C3 is a novel suppressor of cell differentiation that provides a plausible target for the non-cyclooxygenase-dependent antineoplastic actions of nonsteroidal anti-inflammatory drugs. Cancer Res. (63), 2003, 505−512.
38. J. Komoto, T. Yamada, K. Watanabe, D. F. Woodward, F. Takusagawa, Prostaglandin F2alpha formation from prostaglandin H2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost. Biochemistry (45) 2006, 1987−1996.
39. T. Yoda, K. Kikuchi, Y. Miki, Y. Onodera, S. Hata, K. Takagi, Y. Nakamura, H. Hirakawa, T. Ishida, T. Suzuki, N. Ohuchi, H. Sasano, K. M. McNamara, 11beta-Prostaglandin F2alpha, a bioactive metabolite catalyzed by AKR1C3, stimulates prostaglandin F receptor and induces slug expression in breast cancer. Mol. Cell. Endocrinol. 413, (2015), 236−247.
40. A. Mantel, A. Carpenter-Mendini, J. VanBuskirk, A. P. Pentland, Aldo-keto reductase 1C3 is overexpressed in skin squamous cell carcinoma (SCC) and affects SCC growth via prostaglandin metabolism. Exp. Dermatol. 23, (2014), 573−578.
41. B. Wang, Y. Gu, K. Hui, J. Huang, S. Xu, S.Wu, L. Li, J. Fan, X. Wang, J. T. Hsieh, D. He, K. Wu, AKR1C3, a crucial androgenic enzyme in prostate cancer, promotes epithelial-mesenchymal transition and metastasis through activating ERK signaling. Urologic Oncology: Seminars and Original Investigations, 36(10), (2018), 472.e11-472.e20. https://doi.org/10.1016/j.urolonc.2018.07.005.
42. S. M. Dehm, D. J. Tindall, Prostate Cancer: Cellular and Genetic Mechanisms of Disease Development and Progression; Springer: Cham, Switzerland, (2019).
43. Tucci, M.; Caffo, O.; Buttigliero, C.; Cavaliere, C.; D'Aniello, C.; Di Maio, M.; Kinspergher, S.; Maines, F.; Rizzo, M.; Rossetti, S.; et al. Therapeutic options for first-line metastatic castration-resistant prostate cancer: Suggestions for clinical practise in the CHAARTED and LATITUDE era. Cancer Treat Rev. 74, (2019), 35–42. https://doi.org/10.1016/j.ctrv.2019.01.002.