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Resveratrol: An Anti-Androgen for the Treatment of Prostate Cancer

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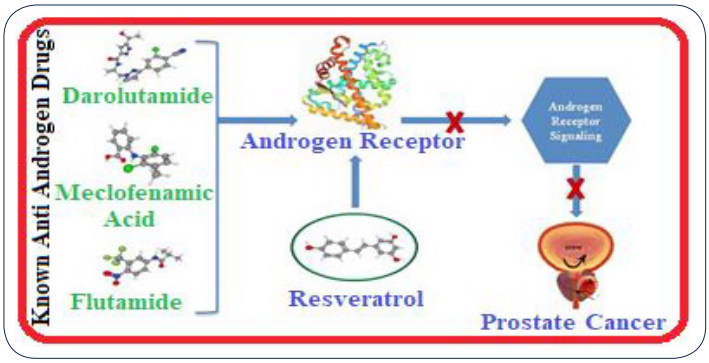
Abstract

Androgen Receptor (AR) is a transcriptional factor for testosterone and 11 Dihydrotestosterone (DHT) that mediates the biological responses of androgens in the 12 prostate gland and it plays pivotal roles in prostate cancer. This study is aimed at 13 investigating possible inhibitory effects of Resveratrol on androgen receptor. It also draws a 14 comparison of binding affinity of Resveratrol with some known anti-androgenic drug with 15 the help of an in silico approach. The molecular Docking and binding free energy 16 calculation studies show the molecular interaction of the anti-androgenic compounds with 17 human Androgen Receptor (AR) which may interfere in the androgen signaling in human. 18 Primary results from computational approaches revealed that the compounds bind 19 selectively to AR with significant potential to affect Androgen signaling in humans. 20 Another noteworthy highlight of this research would be the analysis of Resveratrol's 21 binding behavior as Resveratrol has managed to beat Flutamide (a known drug for prostate 22 cancer) comprehensively in terms of its docking score and binding affinity. All the results 23 obtained are conclusive enough that Resveratrol can be used as a potential drug for prostate 24 cancer.

Keywords: Androgen receptor; Anti-androgens; Resveratrol; Prostate cancer; Molecular docking.

Graphical abstract



Article highlights

- Androgen Receptor (AR) plays pivotal roles in Prostate Cancer.
- The study explores the ability of Resveratrol to arrest the development of Prostate Cancer by inhibiting the AR.
- Molecular docking studies established that Resveratrol can strongly bind to AR and can beat Flutamide, a known drug, in terms of its docking score and binding affinity.

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Introduction

Cancer being the menace that has claimed innumerable human lives has several manifestations and routes to target its every victim. Among these, Prostate cancer (Pca) leads the charts in the United States. It is the country's most diagnosed form of cancer which eventually has placed itself as the second leading cause of cancer death in American men [1]. An exploratory study into its root cause and mechanisms suggest enough evidence that androgens, a class of endogenous hormones are at the backdrop of its development. The androgen receptor (AR) belongs to the subfamily of steroid hormone receptors that falls under a larger family of nuclear proteins [2]. AR mediates the actions of male sex steroids. Testosterone and its metabolite, Dihydrotestosterone (DHT), are necessary for the normal growth and maintenance of the prostate gland [3]. However higher serum levels of testosterone, Dihydrotestosterone (DHT), or other androgens has not been consistently seen in men with prostate cancer. Therefore, a clear correlation between AR levels and Pca can be observed.

Charles Huggins, in 1940, suggested that patients with metastatic prostate cancer could be treated by androgen ablation therapy, marking the beginning of prostate cancer therapy [1,4]. With time and medical advancement, a new therapeutic strategy evolved. Chemoprevention today aims to prevent and reduce the risk of cancer through the use of low-toxicity oral preparations of natural or manufactured chemicals that slow, stop, or reverse carcinogenesis [5].

Non steroidal Anti-Inflammatory Medicines (NSAIDs) such as flutamide, indomethacin, piroxicam, and sulindac, which all block cyclooxygenase (COX), may be used as chemopreventive agents [6]. COX catalyses the conversion of arachidonic acid to pro-inflammatory substances like prostaglandin, which is known to stimulate tumour growth and suppress immune surveillance [7]. This inhibitory activity is important for cancer chemoprevention because COX catalyses the conversion of arachidonic acid to pro- inflammatory substances like prostaglandin, which is known to stimulate tumour growth and suppress immune surveillance. COX can also activate carcinogens to restore genetic material that has been damaged [8]. Florence Menegaux, through a population based case–control study, showed that frequent and chronic use of NSAIDs can lower the risk of prostate cancer and targeting chronic inflammation help preventing prostrate carcinogenesis [9].

Fenamates are a class of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) known for their potent anti-inflammatory effects when the COX enzyme is inhibited [10]. At the same time, these drugs are particularly efficient AR inhibitors. Fenamates' antitumor impact is due to the inhibitory mechanisms, therefore they have a lot of potential in cancer treatment [11]. Meclofenamic acid had the greatest therapeutic efficacy of all the fenamates investigated for their positive effect on Pca. At all stages of the disease, agents that target the Androgen Receptor (AR) are utilised to prevent and treat prostate cancer (Pca). Thus, Flutamide and Darolutamide, which are well known drugs for Pca, can inhibit the activity of AR and are also known as anti-Androgens [12]. In search for new cancer chemopreventive agents over the past several years, hundreds of plant extracts have been assessed for their potential to inhibit action of AR [13].

Recently Resveratrol (RSV), a well-known phytoalexin, has been found to show inhibition of cellular events associated with tumor initiation, promotion, and progression [14]. Resveratrol is found in a multitude of dietary plants including grapes, berries and peanuts [15,16]. Various supplements and dietary sources incorporate the cis and trans-isomers of this phenolic compound. However the bioavailability of the trans-form is relatively higher as compared with the cis-form [17,18]. High levels of resveratrol in grape skin are synthesized when subjected to fungal infections [19]. Grape juice, which is also a source of wine, has particularly high concentrations of resveratrol [20].

Li Kai et al observed that treatment of LNCaP cells with resveratrol could result in the inhibition of androgen-promoted growth and lowering of the prostate-specific antigen levels. Resveratrol works in concert with the known anti androgen flutamide to reduce the amount and activity of AR [21].

Resveratrol has been found to improve the apoptosis-inducing potential of TRAIL in prostate cancer cells through several in vitro methods. Resveratrol's ability to prevent tumour development and metastasis while also enhancing the therapeutic potential of TRAIL implies that it could be utilised alone or in conjunction with TRAIL to treat prostate cancer [22].

Iguchi et al investigated the ant androgenic activities of resveratrol analogs in order to 102 identify a potent anti androgen compound as a lead [23].

Resveratrol has displayed a potential as prostate cancer chemoprevention in both in vitro and animal model studies. Despite promising results from many in vivo and in vitro studies, there are no reports of human clinical trials on therapeutic effects of resveratrol in prostate diseases. An in silico study is therefore designed to explore the human prostate cancer preventing ability of both cis and trans forms of resveratrol by inhibiting the AR. The comprehensive computational study has been carried out to superimpose the inhibitory action of Resveratrol over some known AR inhibitors. This way a concerted effort has been made to establish Resveratrol as a potential drug to control Pca through elucidating its mechanism for prostate cancer prevention.

Materials and methods

All computational analyses were carried out using Schrödinger Software Suite 2018-2. Glide software developed by Schrödinger was used for molecular docking studies of the selected anti- androgen compounds with androgen receptor. The structures of the ligands were drawn using Maestro 2D sketcher (Schrödinger). LigPrep module was used for the generation of accurate 3D structures of the molecules and OPLS-2005 was used for energy optimization. The ionization states for all the molecules at desired pH (7.0 ± 2.0) were generated using Epik. The 3D structure of the androgen receptor bound to the native ligand (R-Bicalutamide) with resolution of 2Å was retrieved from protein data bank (PDB ID: 1Z95). The downloaded protein structure was refined using Protein preparation wizard. During structure refinement all missing residues and amino acid side chains were added. Some unrelated parts such as heteroatoms and water molecules were removed from the protein structure retaining the co-crystallized AR inhibitor. An optimized structure of the protein with H-bonding network free from steric clashes was obtained using OPLS-2005 force field.

The fully optimized protein structure was used for the receptor grid generation using Glide software. The ligands were docked to protein grid using Extra Precession (XP) mode. The protein-ligand complex was visualized using Schrödinger Maestro. As ligands can induce conformational changes in the active site of the receptor upon binding, Schrödinger Induced Fit Docking (IFD) protocol was followed.

Binding Affinity calculations were carried out using MM-GBSA methods to estimate relative binding affinities of the ligands to AR with good accuracy. In this study, Prime module of Schrödinger 2018-2 with MMGB-SA was used for binding affinity calculation to evaluate the binding strength of the specific ligands. The binding affinities of the compounds with AR were evaluated with a related post- scoring approach by estimating free energy of binding. The molecular mechanics energies combined with the generalized Born and surface area continuum solvation (MM/GBSA) methods were used to estimate the free energy of the binding.

Results

Molecular docking analysis

Molecular docking study was carried out to reveal the nature of interaction of the five compounds (Figure 1) with the target AR (PDB: 1Z95) and to draw a comparison of binding affinity of Resveratrol with the known anti androgenic drugs such as Darolutamide (DLA), Meclofenamic Acid(MFA) and Flutamide and R-Bicalutamide.

The docking results also provided relevant information about the binding energy and orientation of ligands during binding with the androgen receptor. The docking studies were carried out using the reported structure of AR bound to R-Bicalutamide, a known antiandrogen compound used to treat prostate cancer. The Root Mean Square (RMS) deviation between the actual and the predicted pose was found to be 0.8 Å, which is within the normal acceptable limit of 2.0 Å.

Molecular docking of darolutamide with AR

With a docking score of -14.910 kcal mol-1, the chemical Darolutamide, a known selective competitive antagonist of the androgen receptor, docks efficiently with the AR. The molecule interacts with the receptor in both electrostatic and hydrophobic ways, generating a stable complex. The compound's two -NH moieties were shown to exhibit two H-bonds with THR-877 of the AR. Again, the Darolutamide ligand exhibited three H-bonds with three AR amino acid residues: MET-742, TYR-739, and HIE-874. In addition, 30 amino acid residues were found to have hydrophobic interactions (Figure 2, Figure S1 and Table S1).

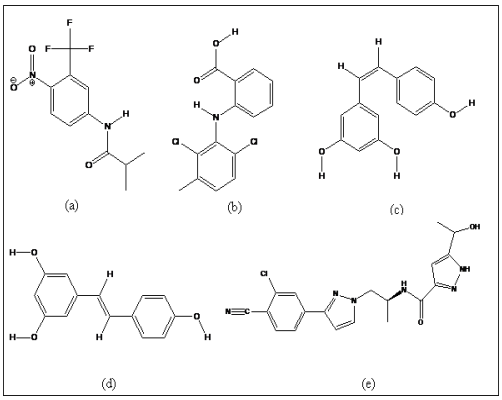

Figure 1: 2D structures of (a) Flutamide, (b) Meclofenamic acid, (c) Cis-RSV, (d) Trans-RSV, (e) Darolutamide.

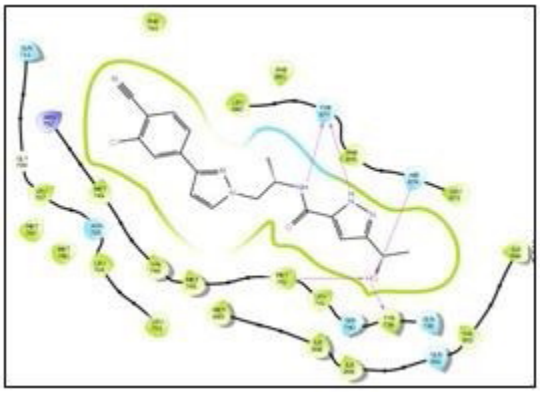

Figure 2: 2D image of the amino-acid residues in the binding pocket of androgen receptor (AR) 387 involved in interactions with Darolutamide.

Table 1: Comparative Molecular Docking score, binding affinity values, no. of hydrogen bonds , 484 number of interacting amino acid residues, and number of common interacting residues during 485 binding of R-Bicalutamide, resveratrol and three anti-androgenic compounds with AR.

Ligands	Docking score (Kcal/m ol)	Binding Affinity (Kcal/m ol)	Glide emodel score	No of H- bonds	IFD score	Number of interacting residues	Number of common interacting residues	Number of interacting residues common with the native ligand (%)
DLA	-14.91	-142.86	-136.142	5	-517.46	30	24	96
R-Bicalutamide	-12.932	-98.86	-97.435	3	-568.66	25	25	100
MFA	-13.464	-88.96	-66.224	2	-566.94	19	16	64
Cis-RSV	-11.875	-85.41	-67.757	5	-566.04	16	13	52
Trans-RSV	-11.198	-92.88	-72.727	3	-565.48	19	15	60
Flutamide	-10.536	-77.66	-52.96	3	-563.59	20	17	68

Molecular docking of maclofenamic acid with AR

Maclofenamic acid can likewise bind well with AR, according to a molecular docking 171 study, with a docking score of -13.464 kcal mol^{-1}. The compound is stable due to a mix of 172 electrostatic and hydrophobic interactions. The AR's GLN-711 and ARG-752 amino acid 173 residues establish two H bonds with the ligand's O^- ion. The AR residue PHE-764 interacts with 174 the aromatic ring of the ligand Maclofenamic acid in a one-interaction. Various hydrophobic 175 interactions were identified between the chemical and the binding pocket's 19 amino acid 176 residues (Figure 3, Figure S2 and Table S1).

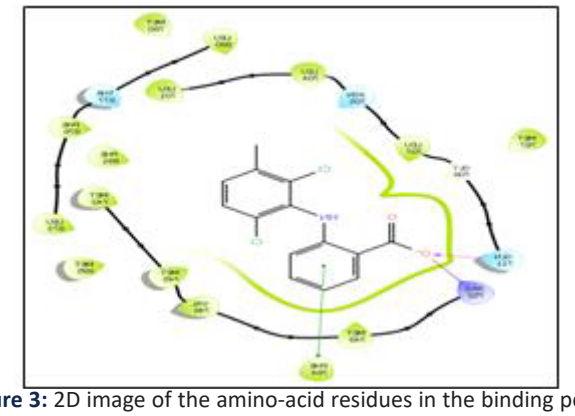

Figure 3: 2D image of the amino-acid residues in the binding pocket of androgen receptor (AR) 408 involved in interactions with Maclofenamic acid.

Molecular docking of Cis-Resveratrol with AR

With a docking score of -11.875 kcal mol^{-1}, the Cis-RSV may likewise bind rather effectively with AR, according to the results of the docking investigation. The complex's stability was achieved by a mix of electrostatic and hydrophobic interactions. The amino acid residues of 183 AR viz. ARG-752, GLN-711, and MET-745 created three H bonds with the ligand's OH group. ASN-705 and LEU-704 make two more H bonds with the ligand's two other OH groups. The AR's PHE-764 residue develops a π-π interaction with the aromatic ring of Cis- RSV. 186 Furthermore, hydrophobic interactions with 16 different amino acid residues in the binding pocket were discovered. (Figure 4, Figure S3 and Table S1).

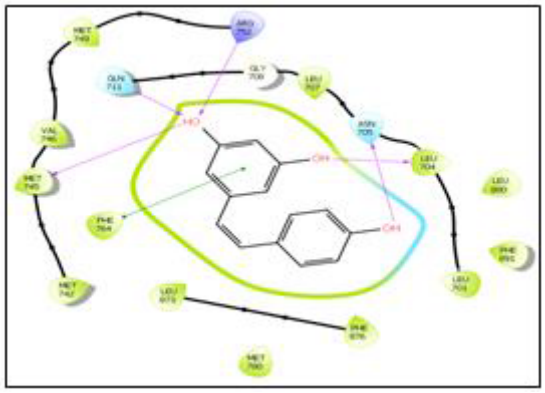

Figure 4: 2D image of the amino-acid residues in the binding pocket of androgen receptor (AR) 425 involved in interactions with Cis-Resveratrol.

Molecular docking of trans-resveratrol with AR

A docking research demonstrated that trans-RSV and AR have remarkably comparable binding abilities. The observed docking score was -11.198 kcal mol-1, and electrostatic and hydrophobic contacts were found, indicating that the complex was stable. The MET-745 and GLN-711 amino acid residues make two H bonds with the ligand's OH group. The ASN-705 formed two more H bonds with another OH group of the ligand. A π-π interaction was discovered between the AR's PHE-764 residues and the ligand's aromatic ring. Different types of hydrophobic interactions were also observed with 19 amino acid residues (Figure 5, Figure S4 and 198 Table S1).

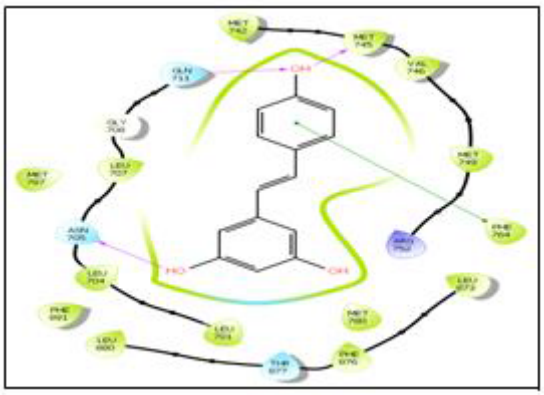

Figure 5: 2D image of the amino-acid residues in the binding pocket of Androgen Receptor (AR) involved in 454 interactions with trans-Resveratrol.

Molecular docking of flutamide with AR

The chemical compound flutamide has a docking score of -10.536 kcal mol^{-1}. A docking investigation demonstrated that it interacts with the receptor in a combination of electrostatic and hydrophobic ways. The GLN-711 and ARG-752 amino acid residues make two H bonds with the ligand's one O- ion. A π-π interaction was discovered between the AR's PHE-764 residue and the ligand's aromatic ring. In addition, 20 amino acid residues were found to have multiple hydrophobic interactions (Figure 6, Figure S5 and Table S1).

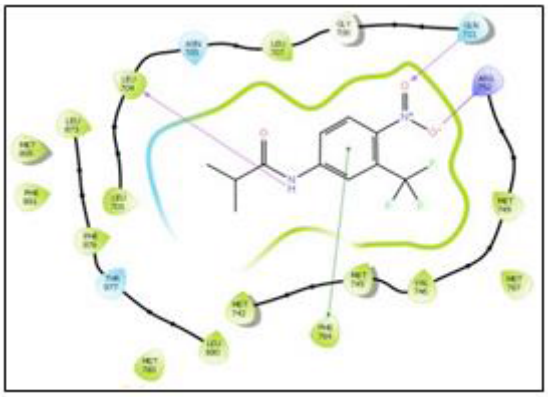

Figure 6: 2D image of the amino-acid residues in the binding pocket of androgen receptor (AR) 474 involved in interactions with Flutamide.

Discussions

Binding free energy calculation

In the field of pharmaceutical research, computational strategies are of great interest because they help in the identification and development of novel and promising compounds especially by molecular docking analysis. Therefore we have carried out this molecular docking approach to evaluate the potential of resveratrol. Binding affinities (MM-GBSA scores) of the compounds were calculated and compared with the score obtained with R- Bicalutamide, a well-known AR blocker. All the results are reported in Table 1. Glide scores and MM-GBSA values obtained after docking of ligands into the catalytic pocket of AR were examined where the ligands Cis and Trans-RSV have shown higher binding affinity towards Androgen receptor compared to flutamide. Moreover, the induced fit docking scores of these two compounds were much higher than that of DLA, another known drug for prostate cancer.

Bicalutamide is one of the Non steroidal Anti androgen (NSAA) compounds used in the treatment of prostate cancer. It works by blocking the Androgen Receptor (AR), the biological target of testosterone and dihydrotestosterone.

The most widely used computational methods in drug design are docking and scoring where by the binding mode of the drug is predicted, followed by an estimate of the binding affinity. The docking score and binding energy comparison clearly indicated that both cis and trans resveratrol can bind to the binding pocket of AR and can form stable complexes. This explains the beneficial effect of this compound in prostate cancer and justifies its use in combination with known anti-androgen compounds to produce a synergistic effect. Therefore it will be worth carrying out further investigations of resveratrol at both in vitro and *in vivo* levels for prevention or management of prostate cancer.

Conclusions

This study was conceptualized with a two-pronged objective: Firstly, it investigates the role of five selective anti-Androgen compounds in prostate cancer treatment and secondly, it draws a comparison of binding affinity of Resveratrol with known anti androgenic drugs (Darolutamide, Meclofenamic acid & flutamide). Molecular docking was deployed as a methodology to perform the investigation. Primary results from computational approaches revealed that the compounds bind selectively to AR with significant potential to affect Androgen signaling in humans. Though both the cis and the trans isomers can bind to AR, the binding affinity of the trans isomer is little higher. Another noteworthy highlight of this research would be the analysis of Resveratrol's binding behavior. While displaying enough binding affinity towards AR, Resveratrol has managed to beat Flutamide (a known drug for prostate cancer) comprehensively in terms of its docking score and binding affinity. However, known anti androgenic drugs like Darolutamide and Meclofenamic acid have shown the best docking score and the binding affinity. A corollary to this study was insights developed into the binding modes and key interactions of the androgen receptor and anti-androgen complexes. Analysis of the components in binding free energy pointed out that the major contributions to the binding process are hydrogen bonding, pi-pi interactions and hydrophobic interactions. All the results obtained conclusively establish Resveratrol as a potential drug for prostate cancer. These findings may aid in a better structural understanding of Androgen receptor modulators in targeting the AR and form a preliminary base for further development of Resveratrol or its derivatives as potential drug for the treatment of prostate cancer.

Declarations

Funding information: No funding is available for this Research.

Conflict of interest: Authors declare no conflict of interest.

Availability of data and material: The data that support this work are available in the article. Table S1 and Figure S1-S5 are given as supporting information material.

Code availability: Not applicable.

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