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Computer aided screening of inhibitors to 5-α reductase type 2 for prostate cancer

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Abstract:
Traditionally, drugs are discovered by testing compounds synthesized in time consuming multi-step processes against a battery of *invivo* biological screens. Promising compounds are then further studied in development, where their pharmacokinetic properties, metabolism and potential toxicity were investigated. Here, we present a study on herbal lead compounds and their potential binding affinity to the effectors molecules of major disease like Prostate Cancer. Clinical studies demonstrate a positive correlation between the extent of 5-α reductase type 2 (isoform 2) and malignant progression of precancerous lesions in prostate. Therefore, identification of effective, well-tolerated 5-α reductase inhibitors represents a rational chemo preventive strategy. This study has investigated the effects of naturally occurring nonprotein compounds berberine and monocaffeyltartaric acid that inhibits 5-α reductase type 2. Our results reveal that these compounds use less energy to bind to 5-α reductase and inhibit its activity. Their high ligand binding affinity to 5-α reductase introduces the prospect for their use in chemopreventive applications. In addition, they are freely available natural compounds that can be safely used to prevent prostate cancer.

Keywords: 5-α reductase type 2, Prostate Cancer, Berberine, Monocaffeyltartaric acid, Docking, ADMET, and Homology modeling.

Background:
Prostate cancer (PC) is a cancer that occurs in a man's prostate gland, it is one of the most common types of cancer in men **[1]**. PC generally does not present any symptoms until it becomes locally advanced or metastatic disease **[2]**. Androgens are essential for the normal development as well as the onset of prostate cancer through their interactions with the androgen receptor (AR) **[3]**, However, androgen depletion is usually associated with the recurrence of prostate cancer, as monitored by rising PSA levels, and this recurrent disease is termed ''androgen independence'' since advanced prostate cancer remains dependent on AR function. The androgen receptor also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is most closely related to the progesterone receptor, and progestins in higher dosages can block the androgen receptor. The main function of the androgen receptor is as a DNA binding transcription factor which regulates gene expression; however, it has other functions as well. Androgen regulated genes are critical for the development and maintenance of the male sexual phenotype. In some cell types testosterone interacts directly with androgen receptors while in others testosterone is converted by 5-α reductase to dihydrotestosterone, an even more potent agonist for androgen receptor activation. Testosterone appears to be the primary androgen receptor activating hormone in the Wolffian duct while dihydrotestosterone is the main androgenic hormone in the urogenital sinus, urogenital tubercle, and hair follicles. Hence testosterone is primarily responsible for the development of male primary sexual characteristics while dihydrotestosterone is responsible for secondary male characteristics **[2]**.

5-α reductase is an enzyme that was first discovered in the male prostate. It catalyzes the conversion of testosterone to dihydrotestosterone, which in turn binds to the androgen receptor and initiates development of the external genitalia and prostate. The gene for 5-α reductase has been mapped to chromosome 5 **[4]**. The isozyme 5-α reductase 2 is transiently expressed in skin and scalp of newborns. Type 2 is the predominant isozyme detectable in fetal genital skin, male accessory sex glands, and in the prostate, including benign prostatic hyperplasia and prostate Aden carcinoma tissues. 5-α reductase 2 (5αR2) is considered predominant in human accessory sex tissue and is responsible for prostate and male external genitalia development **[5]**. Prostate cancer continues to represent a major cause of cancer related mortality and morbidity, despite the much recent research progress in the field of prostate cancer. Since the early studies of 5-α reductases that lead to the advent of androgen deprivation therapy in the 1940s, **[3]** there has been great interest in knowing basic mechanisms underlying prostate cancer initiation and progression, as well as the potential to target these processes for therapeutic intervention. Here, we present a study on herbal compounds and their potential binding affinity to the receptor molecule 5-α reductase.

Methodology:
Computer aided screening: Two dimensional (2D) similarity searches:
On the basis of chemical similarity, 2D search with natural inhibitor testosterone was performed to find new inhibitors. The presence or absence of

common functional groups such as alcohols or ring systems such as pyrimidines was investigated **[6]**. These 5-α reductase inhibitors were selected from natural products based on their inhibition specificity, drug application and quality. In further steps each compound was analyzed for its possible application as a drug. First, we investigated the absorption and permeability using the Lipinski's rule of five, which implies that molecules should contain less than 10 H-bond-acceptors and less than 5 H-bond-donors. The calculated logP value (describes the lipophilic properties) should be less than 5 and the molecular weight should be less than 500 g/mol **[7]**. Any compound violating more than one rule was not considered since it is not a promising candidate for a drug.

Homology modeling for 3D structure of 5-α reductase-2:
The PDB structures of drug targets were not available. So, modeling of the target proteins were performed using MODELLER. A template search has been performed through BLAST and PSI-BLAST programs **[8]**. Global alignment method was used for comparison between the target-template sequences **[9]**. Gaps with variable gap penalty function are included for structural loops and core regions, in order to get maximum correspondence between the sequences. Alignment file for MODELLER was prepared by CLUSTALW **[10]**. Fold recognition was done through mGenThreader, and LOMETS server for fold assignment **[11]**. Energy minimization of generated 3D-model was done through GROMACS (OPLS force field) by using Steepest Descent and Conjugate Gradient Algorithms **[12]**. Parameters like covalent bond distances and angles, stereo-chemical validation, atom nomenclature were validated using PROCHECK and overall quality factor of non-bonded interactions between different atoms types were measured by ERRAT program. RMSD (root-mean-square deviation) and RMSF (Root Mean Square Fluctuation) was calculated for modeled structures. Functionally important residues (Active-site) were identified through comparative result of POCKETFINDER and SURFACE RACER 4.0.ADMETox box was used to analyse the ADME properties of the candidate molecules.

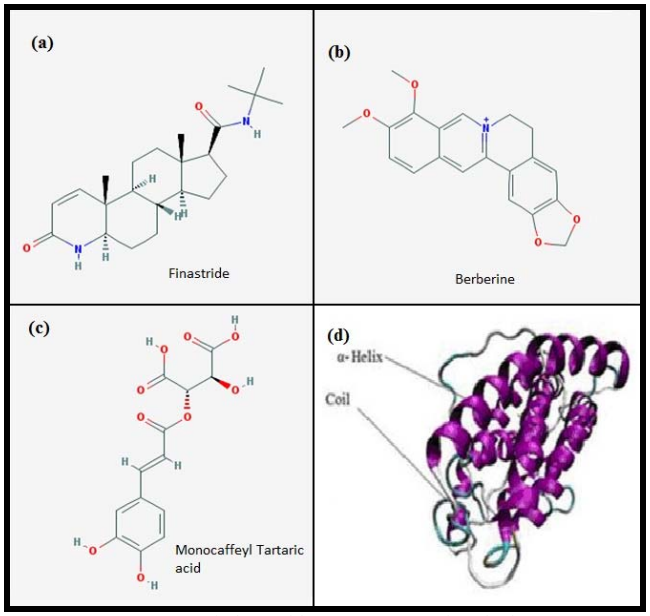

Figure 1: (a) Structure of Finasteride; **(b)** structure of Berberine; **(c)** structure of Monocaffeyltataric acid; **(d)** Modelled Structure using Modeller and Cartoon showing the A- helix and Coil

Result and Discussion:
Around 132 small molecules from different categories such as Alkaloids, Flavanoids, Tannins and glycosides were taken as targeting agents that are responsible for inhibiting the biological processes, important in causing cancer. The investigational drug i.e. Finasteride, which is under clinical trial was used as a reference drug in this study **[13, 14, 15]**. Since 5-α reductase type 2 pathways were mainly responsible for causing prostate cancer, we considered it as our target protein and structure for the same was retrieved through Homology modeling. Modeling of 5-α reductase type 2 was a tedious task due to very low sequence similarity and coverage. Three dimensional model of the drug targets were generated through identified templates along with fold fitting. Fold recognization was done through mGenThreader and LOMETS server for fold assignment. Helices have dominance over other secondary structure i.e. sheet, coil in generated model shown in **Figure 1**. The generated 3D model of target proteins was checked by Ramachandran plot using PROCHECK program. Ala (69), Leu (86) were identified as active site residues. Initial screening of the molecules was based on Lipinski's rule of five. The molecules which satisfy the criteria were subjected to receptor-ligand interaction study using docking tool such as Quantum. Molecules which showed better interactions with 5-α reductase type 2 than Finasteride (reference drug) were considered and subjected to one more docking tool i.e. Quantum, a commercial tool for finding Receptor-ligand interaction and docking score was considered for further result interpretation.

Docking, Binding Site Analysis, and Catalytic active site Analysis:
Protein ligand docking was performed using Quantum 3.3.0 and Hex 4.5. The active site of an enzyme contains the catalytic and binding sites. The structure and chemical properties of the active site allow the recognition and binding of the substrate. The Gbind scores from docking of our chemopreventors were compared to our standard drug (finasteride) and screening was done further to narrow our search for potent inhibitor of 5-α reductase type 2 **(Figure 2b, 2c)**.

Post Docking Study Analysis:
The receptor-ligand complex of the molecules was subjected to active site analysis using SwissPDBviewer (Version 4.0.1) to find the amino acids contributing for binding pocket. The Finasteride, an investigational drug for 5-α reductase type 2 is interacting with Methionine-222, Leucine-42 and Glutamine-224 in terms of hydrogen bond. The Berberine is interacting with Leucine-154 and Isoleucine-182 whereas Monocaffeyltartaric acid is interacting with Aspargine-144, Methionine-141, and Isoleucine-128. The hydrogen bonds of all complexes were located using this tool. **Table 1 (see Supplementary material)** shows the binding site results along with the Hydrogen bond distance, IC50 (calculated using Quantum 3.3.0) for Berberine and Monocaffeyltartaric acid. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}) **[16]**. IC50 value for standard drug (Finasteride) is calculated to be $3.64e^{-001}$ whereas the values for natural compounds: Berberine=$9.71e^{-001}$ and Monocaffeyltartaric acid = $3.05e^{-001}$

(a)

S.No	Compound Name	Source	Score
1	Berberine	Oregon grape	-29.58
2	cichoric	Comfrey plant,Cow urine	-32.11
3	Cynarin	Echinacea	-32.47
4	Delphinidin 3-rutinoside	Ribes nigrum	-30.13
5	Docosahexaenoic acid	Flaxseed oil	-31.74
6	Galetanin	Yellow dock	-35.84
7	Leucocyanidin	Cinnamon,Cocoa,grape seed,grape skin	-27.34
8	Monocaffeyltartaric acid	Echinacea species	-31.06
9	Phytic acid	Sesame seed oil	-47.08
10	Procyanidin	Cinnamon,Cocoa,grape seed,grape skin	-29.58
11	Stearic acid	Fish oil,Palm oil,Olive oil	-28.59
12	Triterpenoide	Gota-Kola	-28.42
13	Finasteride(standard drug)		-26.84

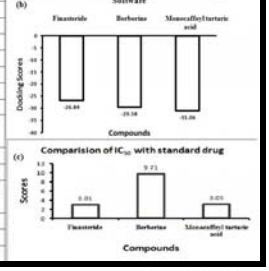

Figure 2: (a) Natural inhibitors of 5α reductase typeII; **(b)** Results of docking using Quantum program; **(c)** Comparison of IC50 of natural compounds with standard.

ADME and toxicity analysis:
Twelve major pharmacokinetics and pharmacodynamics features were predicted for molecules which showed good interaction with 5-α reductase type 2. Moreover, the important features like bioavailability, solubility, drug plasma binding protein and volume of distribution was considered for comparison studies. Toxic effects of molecules were predicted solely from the chemical structure. The ADME and Toxicity properties were predicted using ADME Box and TOX Box tool (http://pharmaalgorithms.com/webboxes/). Tox Box employs large and validated databases, robust Structure-Activity Relationship (QSAR) models in combination with expert knowledge in organic chemistry and toxicology. AMES test parameter was used for finding mutagenicity of the molecules. Health effects in blood, cardiovascular, gastrointestinal system, kidney, liver, and lungs were predicted. **Table 1 (see supplementary material)** shows the ADME and toxicity properties of molecules and Finasteride. It has been found that among all the top twelve small molecules showing better docking score lower than Finasteride (reference drug), Berberine and Monocaffeyltartaric acid were non-commercially available

hence both Berberine and Monocaffeyltartaric acid were considered as a better lead compound than our reference drug (Finasteride) **(Table 2 see Supplementary material)**. Further, ADME-TOX results predict that the Berberine and Monocaffeyltartaric acid natural compounds have lower toxicity than the reference drug. The above synthetic Finasteride inhibitors, prevent or slow down the growth of cancer by dihydrotestosterone suppression **[13]**, and in turn reduce the prostate size **[16]**. The natural compounds, Berberine and monocaffeyltartaric acid (Flavonoid) are found to be the major phenolic constituents in flowers, roots, leaves and involucral bracts and also in the medicinal preparations tested **[17]**. Berberine is a quaternary ammonium salt from the group of isoquinoline alkaloids, derived from tyrosine L- DOPA. It is found in such plants as Berberis, goldenseal (Hydrastis canadensis), and Coptis chinensis, usually in the roots, rhizomes, stems, and bark. Berberine prevents and supresses proinflammatory cytokines. Berberine is an alkaloid derived from tyrosine, L-DOPA. Berberine has drawn extensive attention towards its antineoplastic effects. It seems to suppress the growth of a wide variety of tumor cells including breast cancer, leukemia, melanoma, epidermoid carcinoma, hepatoma, oral carcinoma, tongue carcinoma, glioblastoma, prostate carcinoma, gastric carcinoma. Animal studies have shown that berberine can suppress chemical-induced carcinogenesis, tumor promotion, tumor invasion, prostate cancer, neuroblastoma, and leukemia. It is a radio-sensitizer of tumor cells but not of normal cells. Berberine seems to act as an herbal antidepressant and a neuroprotector against neurodegenerative disorders.

Conclusion:

Our study further confirms that computer aided drug screening is an effective alternative for identification of lead compounds. Several natural lead compounds were identified and tested using molecular docking for their effectiveness against prostate cancer. Berberine and monocaffeyltartaric acid were identified to be effective inhibitors that have the ability to bind to 5-α reductase type 2. Their binding energies were also found to be lower than finasteride. Our results contribute to understanding the mechanisms to explain previous experimental observations and may provide a lead into anticancer research.

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Supplementary material:

Table 1: Entire details of 2 molecules out of 12 screened compounds that follow lipinsky rule

S. No	Compound name	Compound source	Docking score		Neighboring amino acid	H-bond interaction	Ic50
			HEX	QUANTUM			
1	Finasteride(std drug)		-211.88	-26.84	Met222,glu224,leu42	1.17,1.32,1.91	3.64e-001
2	Beberine	Oregon grape	-178.45	-29.58	Leu154,ile54	3.24;5.12	9.71e-001
3	Monocaffeyltartaric acid	Taraxacum afficinale	-159.57	-31.06	Asn144,met141,ile128	1.08,2.6o,2.64	3.04e-001

Table 2: ADMET properties of the compounds

S. No	Molecules	ADME properties					Toxicity properties					
		Oral Bioavailability	Solubility	Drug binding to plasma protein	Volume of Distribution	AMES	B*	C*	Health effects G*	K*	Li*	Lu*
1	Finasteride	%F(Oral)>30%:0.95 %F(Oral)>70%:0.773	-4.03	%PPB:89.81% logKaHSA:3.71	1.97 L/Kg	0.001	0.82	0.91	0.65	0.92	0.94	0.98
2	Berberine	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-6.56	%PPB:25.86% logKaHSA3.15	2.54 L/Kg	0.644	0.50	0.97	0.95	0.99	0.98	0.91
3	Cichoric	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-3.78	%PPB:87.02% logKaHSA4.43	0.23 L/Kg	0.088	0.87	0.46	0.97	0.29	0.89	0.24
4	Cyanin	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-3.73	%PPB:88.61% logKaHSA4.50	0.25 L/Kg	0.034	0.99	0.91	0.99	0.55	0.92	0.87
5	Delphinidi 3-rutinoside	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-5.90	%PPB:54.23% logKaHSA1.55	0.82 L/Kg	0.026	0.99	1.00	1.00	0.88	0.98	0.62
6	Docosahexenoic acid	%F(Oral)>30%:0.854 %F(Oral)>70%:0.450	-4.26	%PPB:99.97% logKaHSA6.06	0.52 L/Kg	0.996	0.97	0.11	0.05	0.84	0.79	0.89
7	Gallotannin	%F(Oral)>30%:0.033 %F(Oral)>70%:0.009	-2.90	%PPB:99.90% logKaHSA3.40	0.58 L/Kg	0.037	0.95	0.95	0.99	0.95	0.74	0.93
8	Leucocyanidin	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-3.16	%PPB:62.39% logKaHSA2.70	1.11 L/Kg	0.020	0.74	0.74	0.81	0.76	0.86	0.78
9	Mono-caffeyltartaric acid	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	-1.41	%PPB:33.76% logKaHSA3.38	0.25 L/Kg	0.071	0.31	0.17	0.34	0.07	0.32	0.29
10	Phytic acid	%F(Oral)>30%:0.033 %F(Oral)>70%:0.008	2.94	%PPB:0.oo9% logKaHSA3.71	0.30L/Kg	0.010	0.26	0.99	0.01	0.84	0.49	0.65