JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34830

SHORT COMMUNICATION

In Silico Screening of Phytochemicals as an Approach against Tubulin Inhibitor in Prostate Cancer

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Abstract

Background: Millions of men worldwide are affected by the complicated disease of prostate cancer, which is most common in areas with high socioeconomic indices. There is growing proof indicating that not all cases of prostate tumors are the same as well as that monitoring techniques and prostate-specific localization therapies are harmless methods of dealing with this mild illness. Plant-based chemicals are believed to be an important reservoir of novel bioactive compounds with a range of different chemical motifs. **Aim:** The potential of tubulin-targeting medications to target Micro tubulin mechanisms and disrupt important cellular processes which include mitosis, cell signalling, cytoplasmic trafficking and angiogenesis is what makes them such effective cancer therapies. **Methods:** The current research uses a variety of applications for emphasizing the drug-like properties, toxicology testing, and *in silico* analysis of numerous phytochemicals in prostate cancer by using the NPACT (Naturally occurring plant-based anti-cancer compound) data bank, Auto dock, Biovia discovery studio for the preparation of target protein and interaction between targeted protein site and Phytocompounds. **Result and Conclusion:** outcomes of the *in silico* screening, such as the discovery of possible bioactive substances or interested targets. The nine phytochemicals exhibited the greatest docking results, proving they are potent inhibitors of prostate tumors. To verify the computational results, compare the *in silico* assumptions with empirical information or previously published literature. To reinforce the conclusions, illustrations such as modifications to conformation, binding ways, or sequences of interactions. This *in silico* study is a critical first step in realizing the enormous promise of plant-based constituents in the field of drugs.

Keywords: Docking, *In Silico*, NPACT, Prostate Cancer, Tubulin, Virtual Screening

1. Introduction

Through the use of natural poisons and traditional remedies, medicine and Natural Products (NPs) have been intimately associated for thousands of years. A database of naturally occurring chemicals generated from plants with anti-cancerous properties is called NPACT. It has 1574 records, each of which includes details on the compound's structure, physical, elemental, and topological characteristics, cancer kind, cell lines, inhibition levels (The IC_{50}, ED_{50}, EC_{50}, GI_{50}), biological targets, commercial providers, and resemblance[1,2]. A gland in men's bodies located just in front of the rectum and underneath the bladder is known as the prostate. Both connective and epithelial tissues make up this structure. It's the tissues assist force semen into the urinary tract while also adding fluid to the semen. Prostate cancer has become more common, and it is no longer possible to distinguish it from other forms of urinary blockage, which has resulted in notable improvements in recognition since the early 1900. Unlike every other cancer form, prostate tumor increases in frequency with age.

2. Types of Prostate Cancer

It determines which sort of cell the malignancy began by looking at the form of prostate cancer.

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Article Received on: 23.08.2023 Revised on: 26.02.2024 Accepted on: 08.03.2024

2.1 Adenocarcinoma

This is regarded as a subtype of carcinoma. It is a specific sort of malignant tumor that may impact many body parts since it develops within the glands that line organs. In glandular epithelial cells, it develops. Hereditary Non-Polyposis Colorectal Cancer (HNPCC), commonly known as Lynch syndrome, and inherited genetic abnormalities such as those in the BRCA1 or BRCA2 genes are risk factors for the illness. Prostate adenocarcinoma symptoms include:

- Urinating often, especially at night.
- Inability to drain completely the bladder.
- Urine with blood
- Irregular erection
- Lying down hurts because of prostate enlargement.
- While excreting, it hurts/burns

There are two types of this prostate cancer:

- Acinar adenocarcinoma of the prostate
- Ductal adenocarcinoma of the prostate

2.2 Stages of Adenocarcinoma

Stage 0: The tumor is still present where it first appeared and has not spread to surrounding tissue.

Stage 1: Because it is localized and tiny, the tumor is not spreading to any closest lymph nodes/organs.

Stage 2: Although tumorous cells have not yet reached distant organs, they have more thoroughly expanded into adjacent tissue and probably local lymph nodes.

Stage 3: It's possible that the tumor is bigger than it was in the second stage, or that lymph nodes or more deeply embedded tissue have cancerous cells present.

Stage 4: The malignancy has spread to additional areas of the body outside of the primary spot. Metastatic adenocarcinoma is another name for fourth-stage adenocarcinoma.

2.3 Transitional Cell Carcinoma

Begins in those cells that line the passageway that carries urine. This form of malignant tumor often develops in the urinary tract and extends to the prostate gland. However, it is incredibly unusual for it to begin its path in the prostate and then move to the bladder opening and the tissues around it.

The first prostate cancer case was documented in 1853 by J Adams, a surgeon at 'The London Lifestyle', the prevalence at the clinical prostate Healthcare. The prevalence in Asian populations is much lower. These tumors were characterized by their mass and a lack of histopathological differentiation. Individuals who received an indication in a severe phase of their condition would die within one to two years[3]. An entirely novel phase of care began when Charles Huggins discovered in the 1940s that cancer that had spread responded to androgen-ablation treatments. Surprisingly, pharmacological treatment with oral estrogen became the first successful comprehensive care for any malignancy, and androgen ablation is still the most widely used method to treat prostate tumors today[4].

3. Androgen Ablation Treatment

John Hunter discussed changes in the size of prostate glands and testicles over the years in animals. He eventually concluded that there was a direct link connecting the testicles and other sexual organs based on the consequences of castration[5].

Randomized research conducted by the Veterans Administration Cooperative Urologic Research Group that started in the 1960s proved to be significant therapy. It shows substantial heart disease and thromboembolic damage when blood levels of testosterone are decreased by oral estrogen. It also became apparent that progressed prostatic tumors would require more than just androgen ablation—either by estrogen administration—to be fully cured[6].

To interrupt/suppress the synthesis of adrenal androgen, novel ways have been invented between the 1960s and the 1980s. Target tissue androgen interaction (BOX 2)[7]. A temporary spike in blood levels of testosterone is generated by the consumption of LHRH agonists that cause discomfort and disruptive effects. Chronic use of such LHRH agonists (BOX 2) led to inhibitory responses, which suppressed concentrations of LH and FSH, thereby lowering blood testosterone levels. The patients who received regular doses of LHRH agonists observed a 75% fall in blood levels of

testosterone and a substantial drop in cancer-related bone discomfort. Later, many more synthesized LHRH agonists emerged for therapeutic use, such as buserelin, goserelin, leuprolide, and nafarelin[7-9].

Prostate cancer treatments employ LHRH antagonists, which obstruct the LHRH receptor straight away[10]. In advanced prostate cancer, clinical studies have been conducted with drugs such as cetrorelix (Cetrotide), abarelix, orgalutran. There are substances that inhibit the synthesis of androgen and adrenal the formation of steroids. Drugs like aminoglutethimide and subsequently ketoconazole were initially among them and as of now are used as second-line hormones in those who do not respond to LHRH-agonist/androgen ablation therapy[11].

Prostatectomy, radiation treatment, and cytotoxic chemotherapy are a few more forms of treatments that are now in use.

4. TUB (Tubulin Alpha-Beta Dimer, Electron Diffraction)

Latest Advances in the Development of Multiple Targeting Tubulin Blockers for Cancer Chemotherapy. One of the cytoskeletal filament structures found in eukaryotic cells, microtubules participate in the movement of substances within cells and are propelled by proteins that circulate on their surface[12].

The structurally significant subunit of the microtubule that serves as cytoskeletal elements necessary for transport within cells and proliferation of cells in all eukaryotes, is the alpha-beta tubulin heterodimer[13,14].

According to an organism's cell population, the Mechanism of tubulin is shown in Figure 1 that how tubulin is polymerized and depolymerized. A popular antitumor technique is to interfere with Micro tubulin kinetics, and several medications that use this method are clinically efficient against a variety of tumors[15-17].

5. Materials and Methods

5.1 Protein Preparation

Tubulin is a target chosen for prostate cancer. The protein's three-dimensional structure was obtained using the Protein Data Bank. We have downloaded the structure of 1TUB from the protein data bank, which shows no mutations and inbuilt ligands like TXL (Taxotere), GTP (guanosine-5'-triphosphate), GDP (guanosine-5'-diphosphate) are present. A guanine nucleotide, which is exchangeable when bound in the beta subunit, or E site, is bound in tubulin monomers, but it is non-exchangeable when bound in the alpha subunit, or N site[18,19].

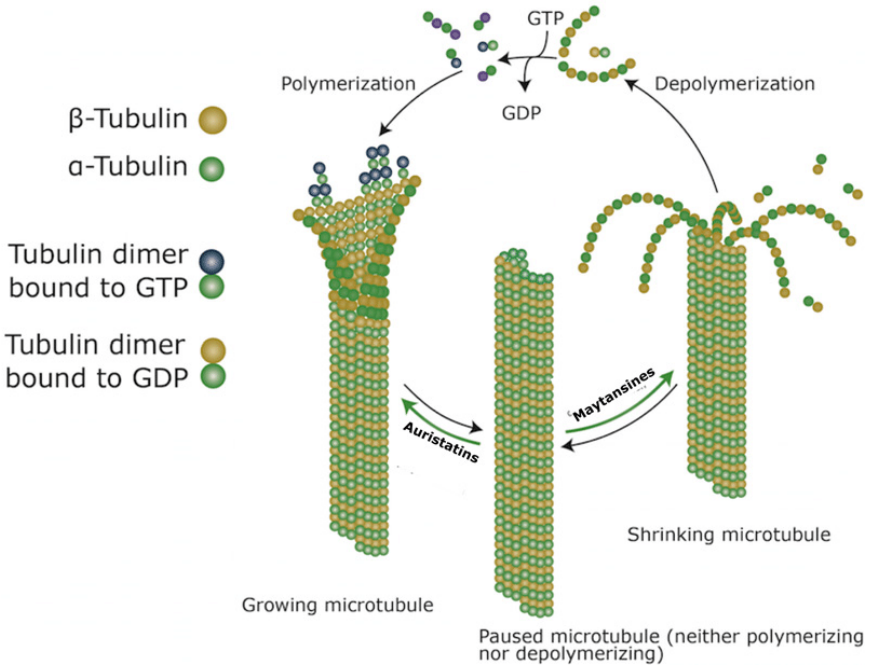


Figure 1. Mechanism of Tubulin[13].

5.2 Dataset Preparation

Compounds were evaluated towards the target for prostate tumors using data gathered from the NPACT Database (naturally occurring plant-based anti-cancerous compound-activity-target). The database's SMILES and SMART designation, framework, and drug-like characteristics are all described, along with its *in vitro* as well as *in vivo* activity. For downloading proteins, the Protein Data Bank was looked into. ChemDraw and Chem3D have been used to create the ligand's three-dimensional framework and to reduce the amount of energy consumed.

5.3 Docking-based Virtual Screening

To evaluate the docking process, Auto-Dock Vina was employed. The missing atoms were repaired, the water molecules were eliminated, the Kollman charges were introduced, and only polar hydrogen was inserted, and so on. Then, the docked ligands were placed in the built-in enzyme's binding region. To visualize interactions, the Discovery Studio Visualizer was used[20].

5.4 Drug-likeness Evaluation

The Lipinski Rule was employed to assess the drug-like qualities of various substances. Every phytoconstituents were examined for qualities that may make it a medication. The Lipinski rule of five was used to assess the drug-likeness characteristics. Furthermore, other factors were also predicted, including GI absorption, Log p, and the absence of a rotatable bond which is shown in Table 1[21,22].

6. Results and Discussion

6.1 Docking Results of *In silico* Study

Globally, more than 1.41 million men are diagnosed with prostate cancer each year. 1 in 7 men will be diagnosed with prostate cancer in their lifetime. US-FDA has approved several medications that have positive as well as negative impacts on patients. They observed adverse effects such as pain and weakening bones, infertility, concentration issues or memory loss, fatigue, infections, and many more. The goal of the ongoing study is to discover substitute natural compounds that can fight prostate cancer by inhibiting tubulin formation.

6.2 Validation of Docking Protocol

Before employing docking to do the dataset's virtual screening, we thoroughly evaluated the Auto-Dock Vina docking process. In Figure 2 structures of the top 9 different scaffolds from virtual screening of phytochemical compounds are shown and their interaction with the defined protein ID (1TUB) with

Table 1. Biological properties using Swissadme

Compound name	Molecular weight	H bond donor	H bond acceptor	Log P	GI absorption	BBB permeability	Bioavailability	Lipinski Violation
1a	270.24	3	5	1.89	High	No	0.55	0
1b	300.26	3	6	2.27	High	No	0.55	0
1c	242.23	0	4	1.76	High	Yes	0.85	0
1d	240.25	0	3	2.24	High	Yes	0.85	0
1e	314.25	3	7	1.92	High	No	0.55	0
1f	302.19	4	8	0.79	High	No	0.55	0
1g	470.6	2	6	3.24	High	No	0.55	0
1h	488.61	3	7	2.93	High	No	0.55	0
1i	472.57	2	7	4.23	High	No	0.55	0

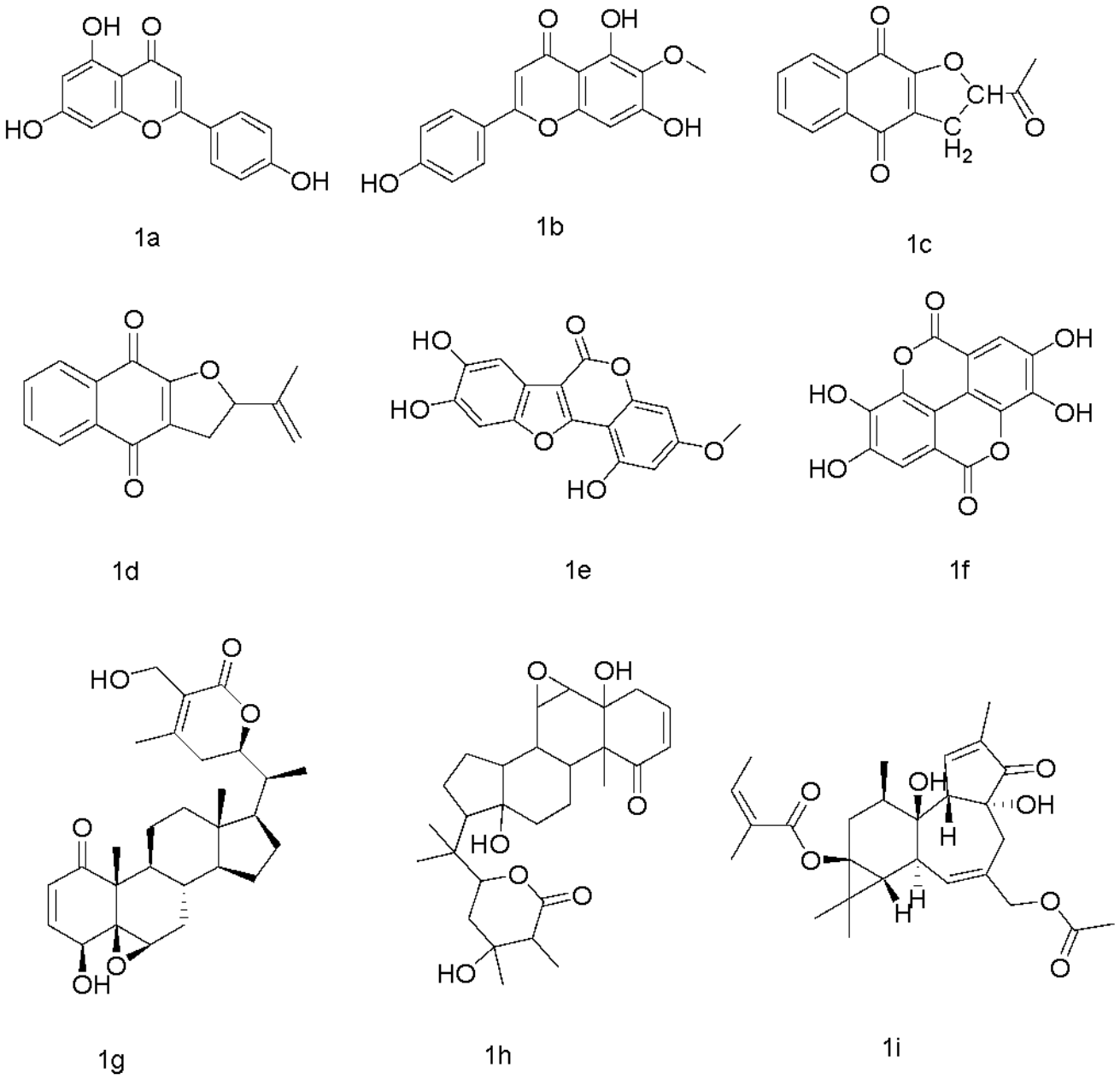


Figure 2. Structures of Top 9 different scaffolds from virtual screening of phytochemical compound.

the use of Biovia Drug Discovery Studio is described in Table 2 and interaction images are demonstrated in Figure 3.

6.3 *In Silico* Toxicity Prediction

Web-based platform ProTox-II, determined the toxicology of the newly identified compounds[23]. The *in silico* toxicological information for each of the defined compounds is shown in Table 3.

7. Limitations of Using Computer-aided Drug Design Methods

The medicinal design is now being accomplished through the application of several computational techniques, but there are also some limitations regarding the use of CADD .methods as follows[24]:

- Difficulties in interpreting the model.

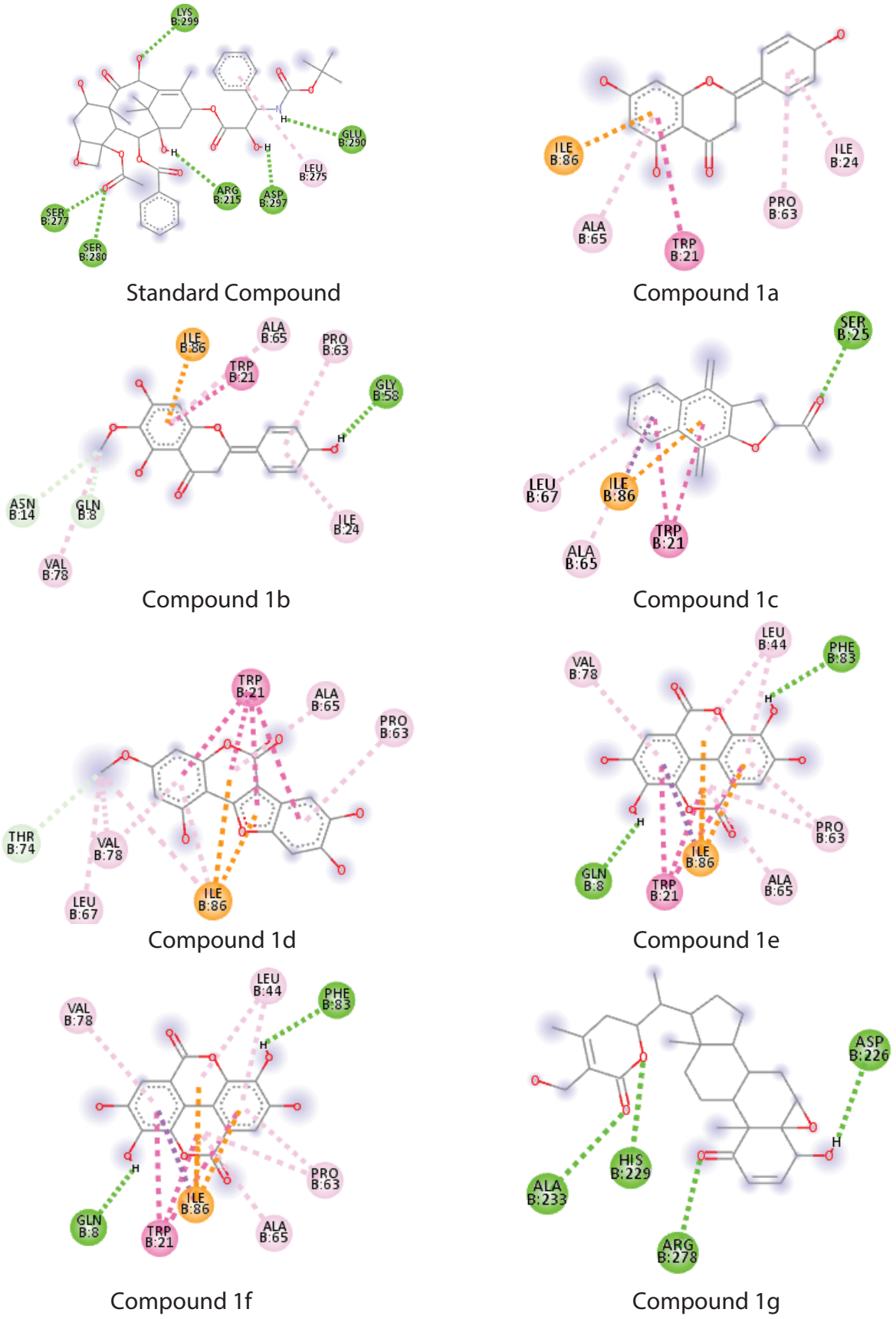
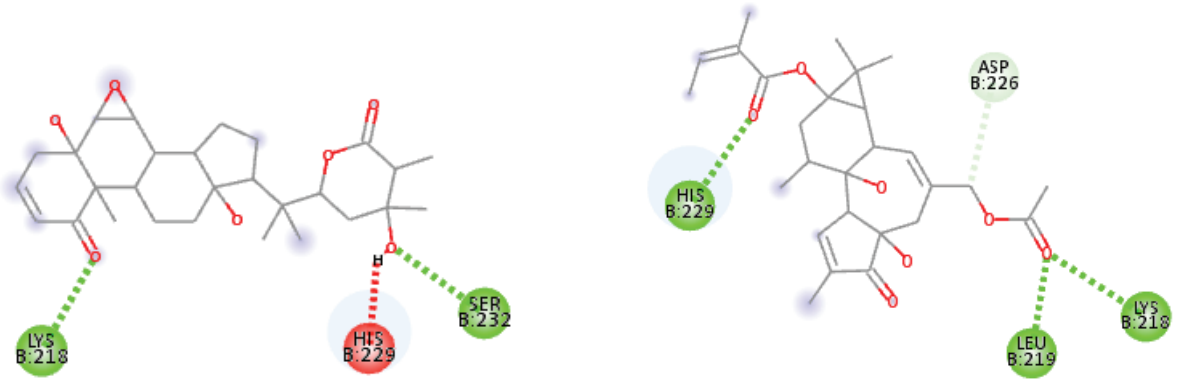


Figure 3. Interaction images of ligands to protein ID (1TUB).



Compound 1h Compound 1i

Figure 3. Continued.

Table 2. Compounds with their binding affinity and amino acid interactions

S. No.	Name	Binding affinity (kcal/mol)	Interacting Residues
*	Standard	-8.0	LYS B:299, GLU B:290, LEU B:275, ASP B:297, ARG B:215, SER B:280, SER B:277
1	1a	-11.6	ILE B:86, ALA B:65, TRP B:21, PRO B:63, ILE B:24
2	1b	-11.8	ILE B:86, ALA B:65, PRO B:63, TRP B:21, GLY B:58, ILE B:24, VAL B:78, GLN B:8, ASN B:14
3	1c	-11.6	SER B:25, TRP B:21, ALA B:65, LEU B:65, LEU B:67
4	1d	-11.3	ILE B:86, PRO B:63, PHE B:83, ILE B:24, TRP B:21, ALA B:65, GLN B:8, VAL B:78, LEU B:67
5	1e	-10.3	ILE B:86, PRO B:63, ALA B:65, ILE B:86, VAL B:78, LEU B:67
6	1f	-10.8	LEU B:44, PHE B:83, PRO B:63, ALA B:65, ILE B:86, TRP B:21, G
7	1g	-10.3	ALA B:233, HIS B:229, ARG B:278, ASP B:226
8	1h	-10.5	LYS B:218, HIS B:229, SER B:232
9	1i	-10.3	ASP B: 226, HIS B:229, LYS B:218, LEU B:219

Table 3. The phytochemical compounds and their toxicological studies (Protox-II)

Name	Hepato-toxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Std	no	no	no	no	no
1a	no	no	no	no	no
1b	no	no	no	no	no
1c	no	no	no	no	no
1d	no	no	yes	no	no
1e	no	no	no	no	no
1f	no	no	no	no	no
1g	no	no	yes	no	no
1i	no	yes	no	no	no

*Inactive – 'No', Active – 'yes'

- Inconsistency in the way the results are tested and validated.
- Inadequately curated database.
- Absence of a cooperative computing paradigm.
- Inadequate function for correct evaluation categorized as in binding affinity, binding mode, forced field SF.
- Issues with multi-domain proteins and the absence of protocols for evaluating the effects of many drugs.

8. Conclusion

Prostate cancer is one of the most common cancers in males globally. Following *in vitro* and *in silico* research, these simulated impacts on outstanding pharmacokinetic and pharmacodynamic attributes could be put into account when planning the initial advancement of therapies against prostate cancer. The nine phytochemicals exhibited the greatest docking results, proving they are potent inhibitors of prostate tumors. The ability of drugs that target tubulin to interfere with crucial cellular functions is what contributes to their being exceptionally strong cancer treatments.

9. References

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