International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

In- Silico Analysis of Structural and Physicochemical Properties of HPV66 E7 Oncoprotein and Its Interaction with Natural Compounds

Namyaa Kumar[1], Dr. Priyanka Narad[2]

[1,2]Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector-125, Noida, India-201313

Abstract

Background: E6 and E7 are the major oncoproteins responsible for the oncogenicity of the Human Papillomavirus in the host- Humans. In this study, bioinformatic tools are used in order to analyse the physicochemical properties of E7 oncoprotein from a high risk HPV strain, HPV-66. Additionally, the study aims to seek potential naturally occurring anti-cancerous compounds which can be effective against cervical cancer through molecular docking studies.

Methods: The physicochemical properties of the both the oncoproteins were predicted and characterized using bioinformatics tool, ExPasy ProtParam tool. Potential anti-cancerous compounds that can be targeted against E7 were identified using literature search. Their ADMET properties were characterised using admetSAR. Molecular docking studies were performed using AutoDock suite 4.2. The docking results were visualized using PyMOL and interaction analysis done using Protein Ligand Interaction Profiler.

Results: HPV-66/ E7 has a molecular mass of 11911.59 Da, theoretical pI value 4.49 and hydrophilicity coefficient GRAVY is -0.338. The study suggests that Bortezomib and Luteolin are promising as potential cervical cancer drugs targeting E7.

Conclusion: The study sheds light on the potential of natural compounds as E7 oncoprotein inhibitors in cervical cancer. Bortezomib and luteolin appear to be promising candidates for further exploration based on ADMET properties and docking information. However, additional research is needed to confirm these findings and develop effective treatments for cervical cancer.

Keywords: Bioinformatic tools, Bortezomib, Cervical cancer, E7 oncoprotein, HPV-66, In-silico analysis, Luteolin

1. Introduction

Cervical cancer is one of the leading causes of cancer mortality among women worldwide [1]. The human papillomavirus (HPV) is the leading cause of cervical cancer, and it is believed that high risk HPV infections cause about 99.7% of cervical malignancies [2]. HPV is a common sexually transmitted virus that can cause a variety of malignancies, including cervical, vaginal, vulvar, anal, penile, and oropharyngeal cancers. It is estimated that around 80% of sexually active women get infected with HPV

at some point in their lives [3]. While the majority of HPV infections are asymptomatic and resolve without medical intervention, persistent infection with high-risk HPV strains might lead to development of cancer. HPV strains are categorized into low-risk (HPV-6, 11, 40, 42, 43, 44, 54) and high-risk HPV types (HPV16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70) [4]. Out of them, HPV16 and HPV18 are frequently implicated in lesions associated with cervical cancer.

HPV type 66 (HPV-66) is a less prevalent but emerging type of HPV that has been linked to the development of cervical cancer. HPV-66 is a high-risk HPV strain, which implies that it has the potential to cause cancer. According to research, HPV-66 is responsible for around 3-4% of cervical cancer occurrences, which is a very high figure considering the global incidence of the disease [5]. Although the global prevalence of HPV-66 infections is low, it is more common in some geographical areas, such as Malaysia [6], Asia and portions of Africa. One of the reasons HPV-66 is classified as an emerging strain of HPV is that it is not protected by the current HPV vaccinations on the market. These vaccines are designed to target the most common high-risk strains of HPV, such as HPV-16 and HPV-18, which account for over 70% of all cervical cancer cases. As a result, there is a need to investigate potential therapy approaches for HPV-66-associated cervical cancer.

When the papillomavirus infects the host, the viral genome is integrated at certain chromosomal sites within the human genome, such as 8q24 and 12q15 [7]. The early region's E1 and E2 proteins are partially or completely inactivated by the integration event. Because one of the functions of E2 is to suppress E6 and E7 production, its inactivation is a critical marker that upregulates onco-proteins E6 and E7. The E7 protein degrades the retinoblastoma protein pRb via the ubiquitin-mediated proteolytic pathway [8], causing cells to enter S-phase of the cell cycle and increase p16 expression. Additionally, HPV oncoproteins participate in other cellular processes such as DNA repair pathway and immune evasion. E7 can also interfere with the host immune response by downregulating MHC class I expression and therefore evading recognition by the immune system [9]. As a result, it is clear that the production E7 viral proteins promotes HPV survival and replication in the supra-basal epithelial layer [10].

Understanding the processes through which HPV oncoproteins contribute to cervical cancer is critical for developing targeted treatments. In-silico docking studies can shed light on the potential of small molecule drugs to suppress HPV oncoprotein activity. By targeting these oncoproteins, it may be feasible to impair their interaction with host cell proteins, hence preventing cervical cancer progression.

The discovery of novel forms of HPV linked to cervical cancer underscores the necessity of ongoing study in this sector. This is especially true in the case of HPV 66, where the epidemiology and pathophysiology of this strain of HPV are less studied. HPV - 66, like other high-risk strains of HPV, is distinguished by the expression of E6 and E7 oncoproteins, which are essential in the development and progression of cervical cancer. As a result, suppression of the oncoproteins E6 and E7 has been identified as a potential therapeutic method for HPV-66-associated cervical cancer. This can be done effectively by the use of in-silico techniques like drug likeliness of compound, ADMET property prediction, high throughput virtual screening, molecular docking, and molecular simulation techniques. In-silico docking studies employ computer-based techniques to estimate a medicinal molecule's binding affinity to a target protein. The concept of this technique is that the binding affinity of a pharmacological molecule and a target protein can be predicted by examining their chemical interactions. They provide various advantages over traditional experimental methods, including the ability to swiftly screen a large number of compounds and the ability to test multiple compounds simultaneously.

An in-silico docking approach was utilized in this study to assess the potential of six small molecule drugs to inhibit the activity of HPV - 66 oncoprotein E7. AutoDock version 4.2, a commonly used software for molecular docking studies, was used for the docking [11].

Bortezomib, Curcumin, Verbascoside, Valproate, Sodium butyrate, and Luteolin were chosen as study compounds. Each of these substances has been proven to have the potential to target HPV-66 E7 oncoprotein.

Bortezomib, is a proteasome inhibitor clinically used for the treatment of multiple myeloma and mantle cell lymphoma. It operates by suppressing the activity of the proteasome, which results in protein degradation within the cell. Moreover, it is responsible for inhibiting and degrading p53, a tumor suppressor protein that is similarly targeted by E6. Several studies have demonstrated that bortezomib can inhibit the proliferation of cervical cancer cells [12, 13]. However, there is no evidence as of now which suggests a direct interaction between E7 and Bortezomib. In this study, we are testing for their possible interaction.

Curcumin, a naturally occurring chemical found in turmeric, has been has also been shown in studies to cause apoptosis and inhibit the migration and invasion of cervical cancer cells. A study found that curcumin has the ability to target the E6 oncoprotein of HPV-16, but no direct interaction with E7 of HPV-66 has been reported [14].

Verbascoside, a phenylpropanoid glycoside, used in the treatment of colorectal cancer and glioblastoma. Verbascoside targets the dimerization of CD44 and hence could be effective against cervical cancer as well.

Valproate is an anti-epileptic medication. According to studies, valproate can cause apoptosis as well as limit the proliferation and migration of cervical cancer cells [15]. Valproate is suspected to be associated with E6 downregulation in HPV-positive cervical cancer cells [16]. However, nothing concrete is available to suggest their possible interaction.

Sodium butyrate is a short-chain fatty acid generated in the colon by bacterial fermentation. It has been demonstrated to have anticancer properties in a variety of cancer types, including cervical cancer. According to studies, sodium butyrate's anticancer effects are due to its capacity to block histone deacetylase enzymes and trigger apoptosis in cancer cells [17]. However, the direct interaction of Sodium Butyrate with E7 is not well documented.

Luteolin is a flavonoid compound which found in a variety of fruits and vegetables including celery, parsley, and chamomile. It has been demonstrated to have a variety of biological activities, including antioxidant, anti-inflammatory, and anticancer capabilities. By regulating several signaling pathways involved in cancer progression, luteolin has been shown to induce apoptosis and limit the proliferation of cervical cancer cells [18].

The six compounds in this in-silico docking investigation were chosen primarily based on experimental in-vitro and in-vivo studies demonstrating their ability to inhibit or degrade oncoproteins associated with cervical cancer. However, the binding mechanism of these compounds with the target proteins has not been fully elucidated yet. As a result, the docking analysis done in this study can provide an insight into the potential interactions that exist between these protein-ligands and can aid in our understanding of their binding interactions. The docking analysis results can also be used to guide future experimental research aimed at discovering and developing novel treatment medicines for HPV-associated cervical cancer.

2. Materials and methods:

2.1. Amino acid sequences

The amino acid sequences of Human Papillomavirus - 66 E7 oncoprotein was obtained from Uniprot. (https://www.uniprot.org)

2.2. Physiochemical parameters

Physiochemical parameters like the isoelectric point, percentage of specific amino acid residues, extinction coefficient and instability index of HPV-66/ E7 was predicted using Expasy tool ProtParam (http://web.expasy.org/protparam/).

2.3. ADMET properties

The ADMET properties of the selected small molecules were predicted using admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) along with other parameters like Subcellular localization, water solubility, carcinogenicity etc.

2.4. Molecular docking

2.4.1. Selection and preparation of Ligands:

The set of ligands used to perform the molecular docking studies against the proteins were selected via thorough literature survey of natural active compounds. Their structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). The SDF files were converted into PDB files by OpenBabel. Further, the selected set of ligands was then further prepared using AutoDock 4.2.

2.4.2. Protein Preparation:

The structures for the chosen oncoprotein targets were obtained through PDB. The protein molecule, E7 was then energy minimized using Swiss PDB Viewer. Further, these proteins were prepared for docking by AutoDock 4.2.

2.4.3. Molecular Docking:

Grid generation: As the E7 oncoprotein of HPV - 66 are less studied, their active sites are not reported. Therefore, blind docking was performed for this analysis. Grid was generated using AutoDock 4.2 centered around the ligand molecules.

Docking: The prepared ligand conformers were then docked in the generated grids for the drug targets. The docking was done using AutoDock 4.2. The algorithm used to perform these docking studies was Genetic Algorithm.

2.4.4. Visualization and analysis of result:

To further analyze the molecular docking results, the generated DLG files were converted into PDB files, of the best conformation, using Python Molecular Viewer 1.5.7. The PDB files were then visualization using PyMOL and further using Protein Ligand Interaction Profiler (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index).

3. Results

3.1. Amino acid sequence of HPV 66/ E7

HPV-66/E7 amino acid sequence as obtained from Uniprot:

MHGKVPTLQEVILELAPQTEIDLQCNEQLDSSEDEDEDEIDHLLERPQQARQAEQHKCYLIHVPCCKCELVVQLDIQS
TKEELRVVQQLLMGALTVTCPLCASSK

3.2. Physiochemical properties of HPV - 66 E7 oncoprotein

HPV-66 E7 oncoprotein comprises of 105 amino acid residues and its molecular mass is 11,911.59 Da. It has a theoretical pI value of 4.49. Its amino acid composition is given in table 1. The total number of positively charged residues (Asp + Glu) is 20 and the total number of negatively charged residues (Arg + Lys) is 8. It comprises of 1661 atoms in total and its empirical formula is $C_{508}H_{834}N_{140}O_{170}S_9$. The instability index is 72.49. It has a hydrophilicity coefficient GRAVY= -0.338 and an aliphatic index of 101.14.

Table 1: Amino acid composition of HPV -66 E7 oncoprotein.

Amino acid residue	Frequency	Relative % of composition
Ala (A)	5	4.8%
Arg (R)	3	2.9%
Asn (N)	1	1.0%
Asp (D)	7	6.7%
Cys (C)	7	6.7%
Gln (Q)	12	11.4%
Glu (E)	13	12.4%
Gly (G)	2	1.9%
His (H)	4	3.8%
Ile (I)	5	4.8%
Leu (L)	15	14.3%
Lys (K)	5	4.8%
Met (M)	2	1.9%
Phe (F)	0	0.0%
Pro (P)	5	4.8%
Ser (S)	5	4.8%
Thr (T)	5	4.8%
Trp (W)	0	0.0%
Tyr (Y)	1	1.0%
Val (V)	8	7.6%

3.3. ADMET properties:

The Absorption, Distribution, Metabolism, Excretion and Toxicity properties of the 6 compounds were evaluated using using admetSAR, which is an online server. It also gives information regarding the subcellular localisation, specific receptor binding affinities, skin sensitization etc which might affect the drug likeliness of the compound. The results are summarised in Table 2.

Table 2: ADMET properties of the small molecules selected for the study.

	Bortezomib	Curcumin	Luteolin	Sodium Butyrate	Valproate	Verbascoside
Ames mutagenesis	-	-	+	-	-	-

Acute Oral Toxicity (c)	III	III	II	III	III	III
Androgen receptor binding	-	+	+	-	-	-
Aromatase binding	-	+	+	-	-	-
Blood Brain Barrier	+	-	-	+	+	-
BRCP inhibitior	-	+	+	-	-	-
Biodegradation	-	-	-	+	+	-
BSEP inhibitior	+	+	-	-	-	+
Caco-2	-	-	+	+	+	-
Carcinogenicity (binary)	-	-	-	-	-	-
Carcinogenicity (trinary)	Non-required	Non-required	Non-required	Non-required	Non-required	Non-required
Crustacea aquatic toxicity	-	-	-	-	-	+
CYP1A2 inhibition	-	+	+	-	-	-
CYP2C19 inhibition	-	+	-	-	-	-
CYP2C9 inhibition	-	+	-	-	-	-
CYP2C9 substrate	+	-	-	-	+	-
CYP2D6 inhibition	-	+	-	-	-	-
CYP2D6 substrate	-	-	-	-	-	-

CYP3A4 inhibition	-	-	+	-	-	-
CYP3A4 substrate	+	-	-	-	-	+
CYP inhibitory promiscuity	-	+	+	-	-	-
Eye corrosion	-	-	-	+	+	-
Eye irritation	-	+	+	+	+	-
Estrogen receptor binding	-	+	+	-	-	+
Glucocorticoid receptor binding	-	+	+	-	-	+
Hepatotoxicity	+	-	-	+	+	-
Human Ether-a-go-go-Related Gene inhibition	+	-	-	-	-	+
Human Intestinal Absorption	+	+	+	+	+	-
Human oral bioavailability	-	+	-	+	+	-
MATE1 inhibitior	-	-	+	-	-	-
Mitochondrial toxicity	+	-	+	-	-	-
Micronuclear	+	+	+	-	-	-
Nephrotoxicity	-	-	-	+	+	-
Acute Oral Toxicity	2.834569454	2.146666527	1.997965455	1.309604764	1.349600077	1.976620793
OATP1B1 inhibitior	+	+	+	+	+	+

OATP1B3 inhibitior	+	+	+	+	+	+
OATP2B1 inhibitior	-	-	+	-	+	-
OCT1 inhibitior	-	-	-	-	-	-
OCT2 inhibitior	-	-	-	-	-	-
P-glycoprotein inhibitior	-	+	-	-	-	-
P-glycoprotein substrate	+	-	-	-	-	-
PPAR gamma	-	+	+	-	-	+
Plasma protein binding	0.872191727	0.640498757	1.065997124	0.000787977	0.972955585	0.710833311
Reproductive toxicity	+	+	+	-	-	+
Respiratory toxicity	+		+	-	-	-
skin sensitisation	-	-	-	-	+	-
Subcellular localzation	Mitochondria	Mitochondria	Mitochondria	Plasma membrane	Mitochondria	Mitochondria
Tetrahymena pyriformis	0.189308673	1.008301854	2.056606293	-1.280691981	-0.296596497	-0.08119455
Thyroid receptor binding	-	+	+	-	-	+
UGT catelyzed	+	-	+	-	-	+
Water solubility	-2.702961431	-3.364097402	-2.999373191	-0.8050264	-2.239633316	-1.675347129

3.5. Molecular docking

The consolidated results from the studies have been compiled below. Table 3 summarizes the results in terms of minimum binding energy obtained in the runs and its estimated inhibition constant, Ki.

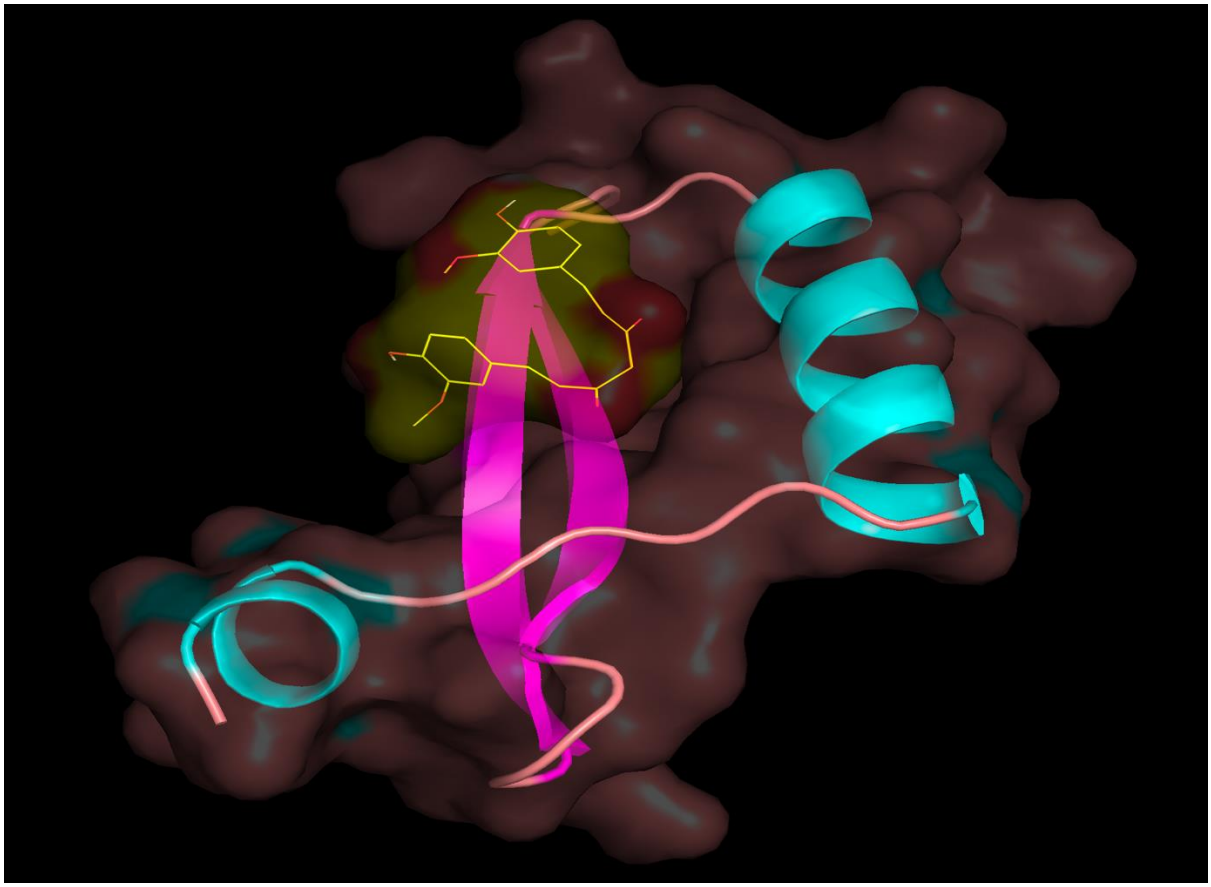
Table 3: Consolidated docking results as obtained from AutoDock 4.2.

Target oncoprotein	Molecule	Minimum binding energy (kcal/mol)	Run	Reference RMSD	Estimated inhibition constant, Ki
E7	L1	-3.19	8	5.8	4.57 mM
	L2	-1.12	2	7.5	149.94 mM
	L3	- 1.45	8	4.5	86.64 mM
	L4	- 3.07	8	4.87	5.57 mM
	L5	- 2.94	4	5.3	7.00 mM
	L6	- 3.14	4	5.64	5.02 mM

Visualization and analysis of docking result:

Only the E7-L1 (E7 - Bortezomib) and E7-L6 (E7 - Luteolin) interactions were studied as they had favorable binding energies. The visualization was done using PyMOL and Interaction analysis was performed using Protein Ligand Interaction Profiler. The results are compiled in Tables 4-7.

Figure 1: a) E7 -Bortezomib docking result visualized by PyMOL; b) Zoomed in view of the interaction.



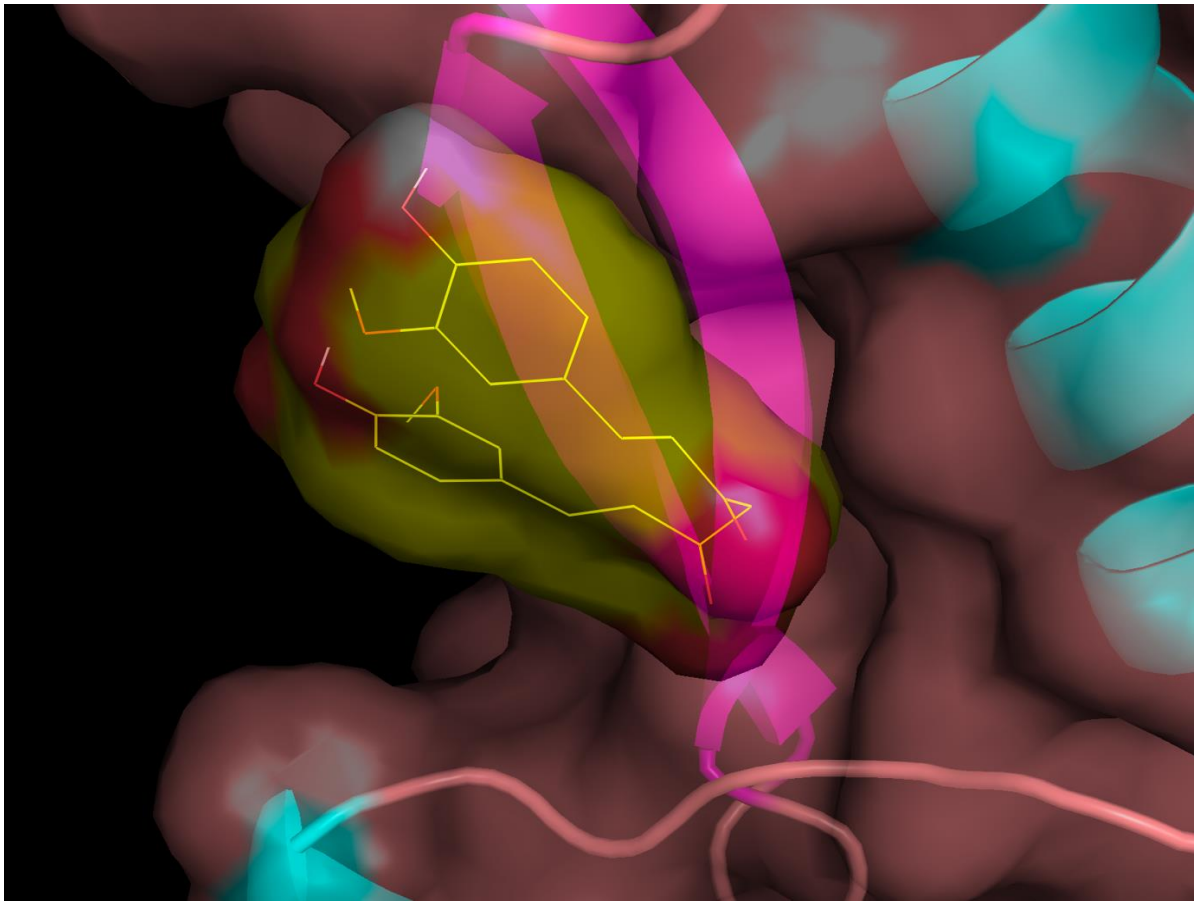
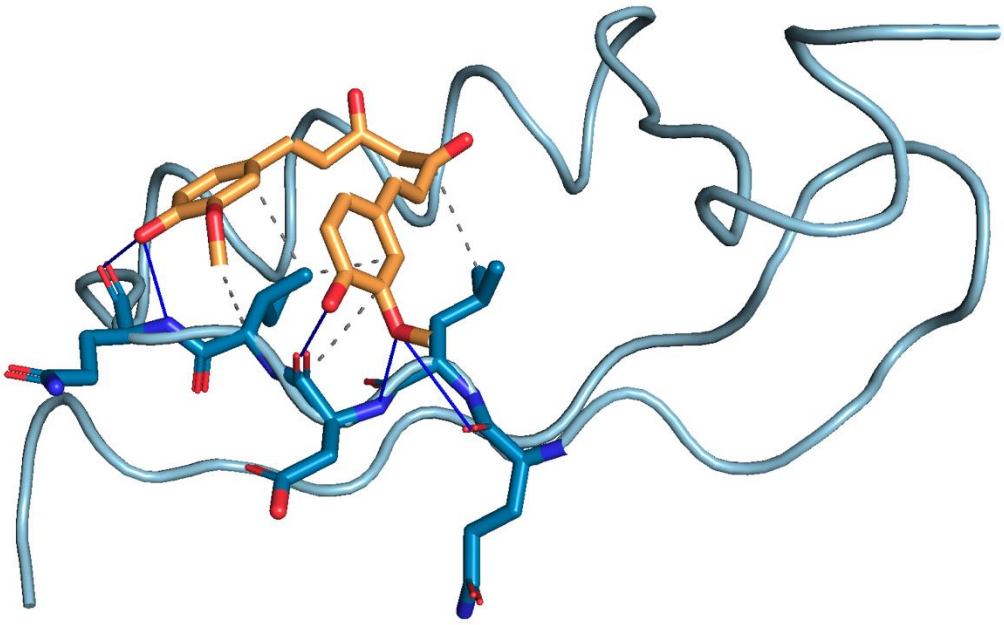
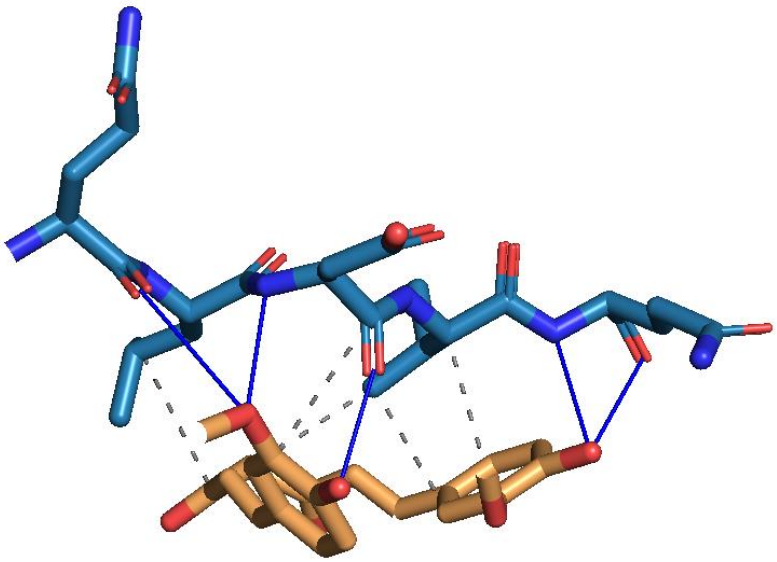


Figure 2: a) Interaction analysis of E7 -Bortezomib docking result visualized by PyMOL; b) Zoomed in view of the interaction





The tables below summarizes the hydrogen bonds and hydrophobic interactions between E7 and Bortezomib.

Table 4: Hydrophobic interactions between E7 and Bortezomib.

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	74A	LEU	3.85	18	224
2	75A	ASP	3.82	16	229
3	76A	ILE	3.5	12	242
4	76A	ILE	3.5	981	242
5	76A	ILE	3.41	979	242
6	76A	ILE	3.29	20	237

Table 5: Hydrogen bonds between E7 and Bortezomib.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	73A	GLN	3.15	3.64	112.6	no	no	27 [O3]	209 [O2]
2	75A	ASP	1.95	2.85	145.23	yes	no	227 [N3]	27 [O3]
3	75A	ASP	2.06	2.81	133.36	yes	no	230 [O3]	29 [O3]
4	75A	ASP	2.16	2.81	123.4	no	no	29 [O3]	230 [O3]
5	75A	ASP	2.16	2.81	123.4	no	no	998 [O2]	230 [O3]
6	77A	GLN	2.02	2.93	146.72	yes	no	245 [N3]	28 [O3]
7	77A	GLN	2.38	2.93	112.65	yes	no	715 [N3]	28 [O3]
8	77A	GLN	1.97	2.83	146.02	no	no	28 [O3]	248 [O2]
9	77A	GLN	1.97	2.83	146.02	no	no	997 [O3]	248 [O2]

Figure 3: a) E7 -Luteolin docking result visualized by PyMOL; b) Zoomed in view of the interaction.

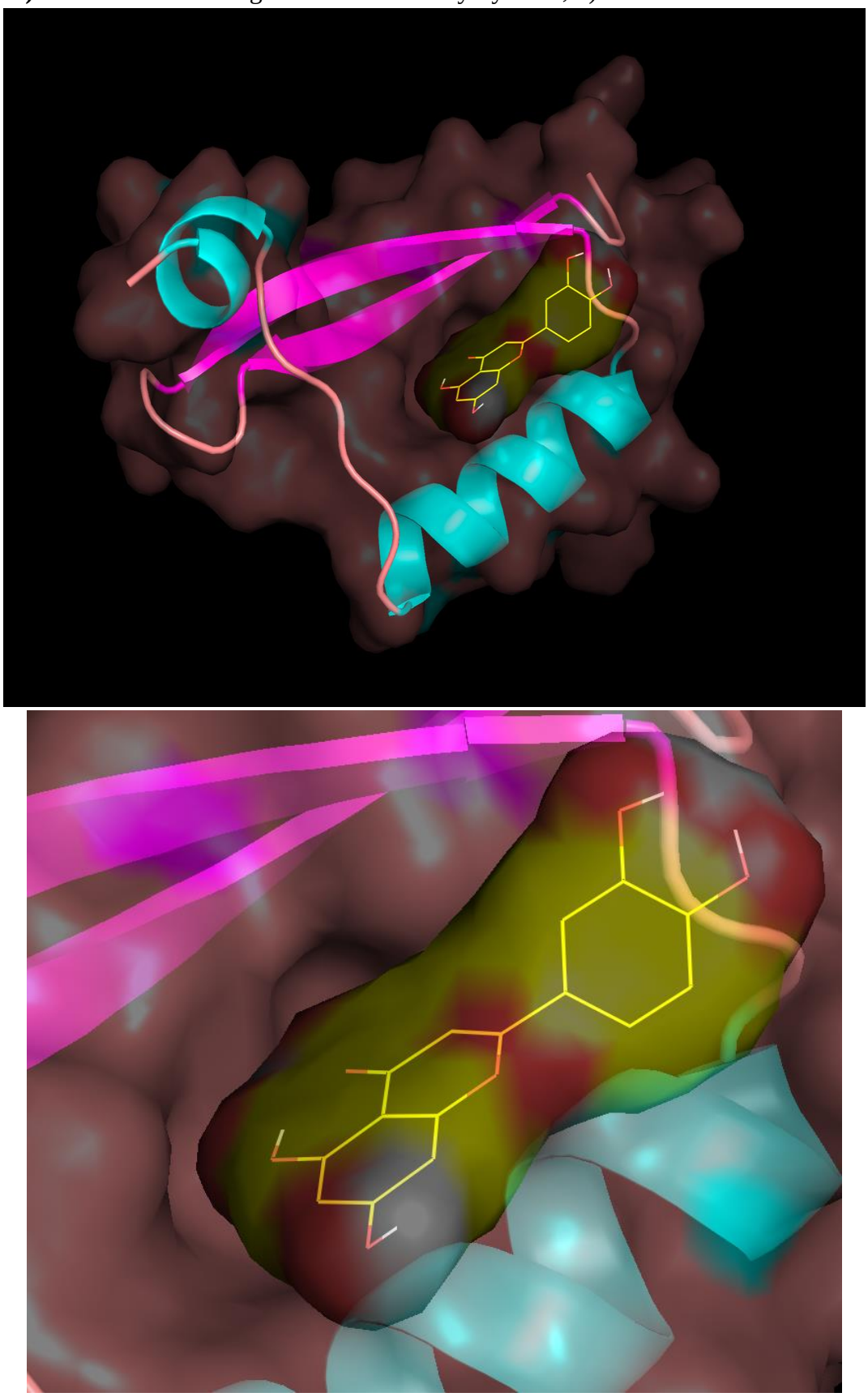
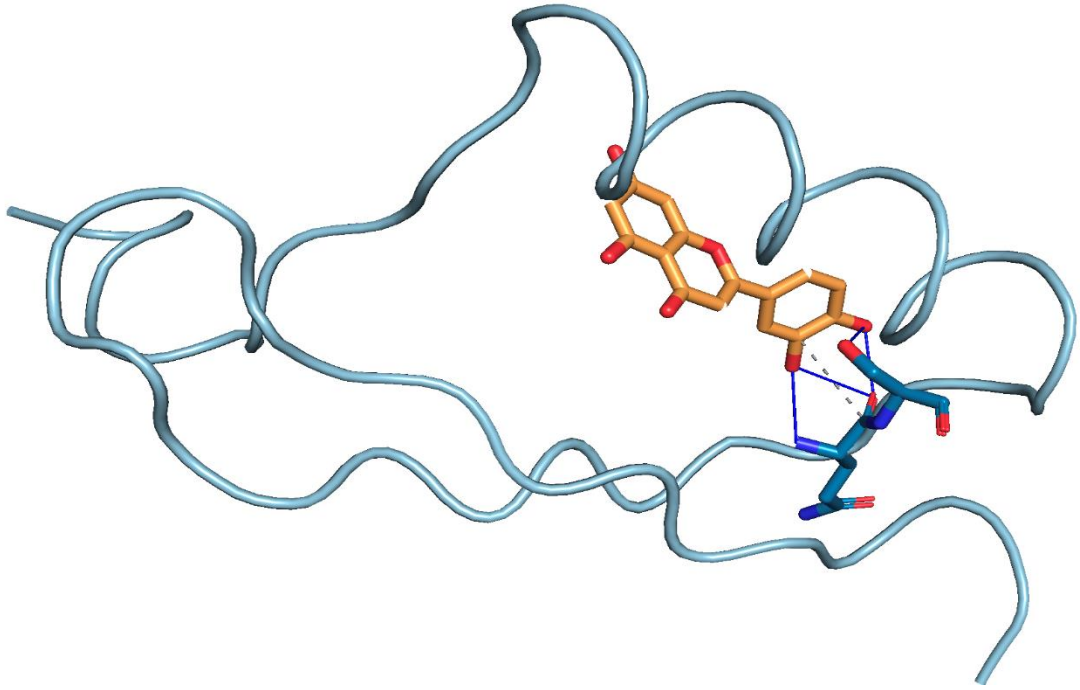
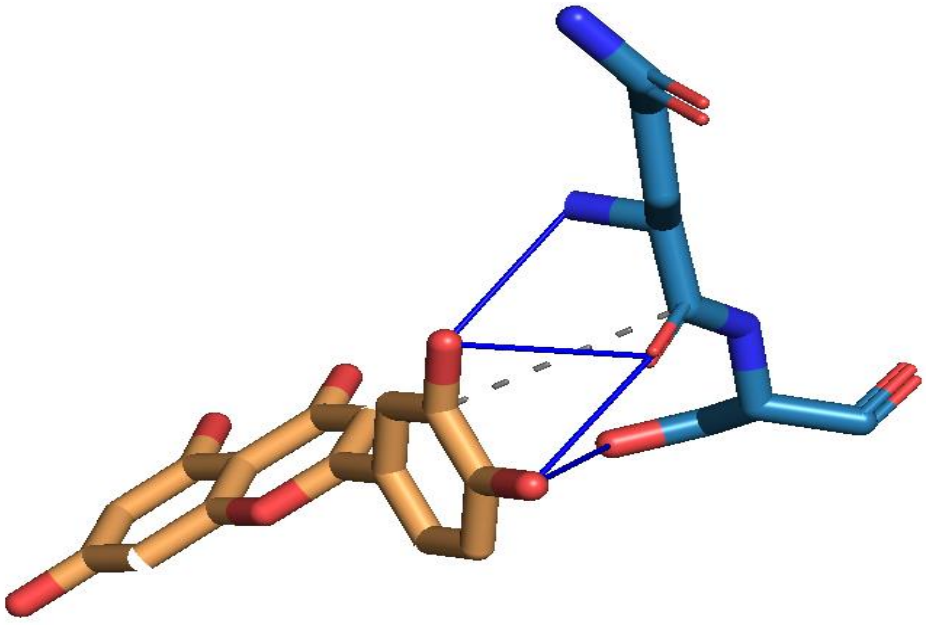


Figure 4: a) Interaction analysis of E7 -Luteolin docking result visualized by PyMOL; b) Zoomed in view of the interaction.





The tables 6 and 7 summarize the hydrogen bonds and hydrophobic interactions between E7 and Luteolin.

Table 6: Hydrophobic interactions between E7 and Luteolin.

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	77A	GLN	3.88	13	243

Table 7: Hydrophobic interactions between E7 and Luteolin.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	77A	GLN	2.14	3.01	142.5	yes	no	711 [N3]	22 [O3]
2	77A	GLN	2.17	2.92	133.09	no	no	22 [O3]	244 [O3]
3	77A	GLN	2.17	2.92	133.09	no	no	987 [O2]	244 [O3]
4	77A	GLN	1.79	2.74	165.1	yes	no	244 [O3]	23 [O3]
5	77A	GLN	2.05	2.74	126.43	no	no	988 [O3]	244 [O3]
6	78A	SER	3.5	3.81	101.31	yes	yes	728 [O3]	23 [O3]

4. Discussion

It is a commonly known fact that the Human Papillomavirus is the prime source of cervical cancer. There are more than 40 types of HPV virus that are disseminative through the genital area. Although many studies are performed on high-risk strains of HPV, very less information is available on HPV-66. Therefore, this bioinformatic analysis was performed on HPV-66 oncoprotein E7 to study its characteristics and interaction with selected compounds.

The amino acid sequence of HPV - 66 E7 oncoproteins was obtained from UniProt. The HPV-66 E7 oncoprotein comprises of 105 amino acids.

The physicochemical properties of HPV 66 E7 oncoprotein was predicted using ProtParam. The molecular mass of the E7 oncoprotein is 11,911.59 Da, the atomic composition is $C_{508}H_{834}N_{140}O_{170}S_9$, the instability coefficient is 72.49, classified as unstable protein and the average hydrophilicity coefficient GRAVY is -0.338 and thus this protein is determined as a hydrophilic protein.

Historically, the significance of natural therapies for diseases has been acknowledged. The importance of finding treatment options that have lesser side effect associated with them can be appreciated without a doubt. A set of 6 such naturally - found compounds were chosen as ligands and were subjected to ADMET prediction and docking studies against the selected drug receptors. Since the structure for the protein used in the study (E7 of HPV 66) is relatively less studied and there was no prior knowledge base for the active binding sites, we used blind docking for this analyzing using AutoDock 4.2.

The ADMET properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of a molecule are crucial in deciding whether it can be developed into a safe and effective drug. The ADMET features of six compounds are discussed in this analysis: Bortezomib, Curcumin, Luteolin, Sodium Butyrate, Valproate, and Verbascoside.

Acute oral toxicity for Bortezomib is rated as III, which denotes a moderate level of toxicity. It does, however, have the ability to pass the blood-brain barrier, which is a significant advantage in treating cervical cancer that has metastasized to the brain. Most CYP enzymes, which are important for the metabolism of numerous medicines, do not inhibit bortezomib. This is a good trait because it decreases the possibility of drug interactions. Bortezomib is not irritating to the eyes, however it causes

hepatotoxicity, or liver damage, and can be absorbed via the human intestine. It is not nephrotoxic, which means it does not harm the kidneys. Bortezomib's subcellular location is in the mitochondria.

Curcumin has a highly hazardous acute oral toxicity rating of III. It does not, however, have the ability to pass the blood-brain barrier. It is inhibited by CYP2C but not by CYP2D6 or CYP3A4. Curcumin is an eye irritant, however it does not cause hepatotoxicity. The human intestine can absorb it, although it is not nephrotoxic. Curcumin's subcellular location is in the mitochondria.

While curcumin has shown limited bioavailability, its formulation as a nanoparticle can help to increase its effectiveness as a drug candidate.

Luteolin has an acute oral toxicity value of II, indicating that it is moderately toxic. It lacks the ability to pass the blood-brain barrier. Most CYP enzymes do not inhibit luteolin, which is a good thing for drug development. Luteolin is an eye irritant, however it does not cause hepatotoxicity. The human intestine can absorb it, although it is not nephrotoxic. Luteolin's subcellular location is in the mitochondria.

The acute oral toxicity rating of sodium butyrate is III, indicating that it is moderately hazardous. It is capable of crossing the blood-brain barrier. Most CYP enzymes do not inhibit sodium butyrate, which is a favorable for drug development. Sodium Butyrate is an eye irritant and causes hepatotoxicity and nephrotoxicity. The plasma membrane is where Sodium Butyrate is found subcellularly.

Valproate is a well-known medication used to treat epilepsy, bipolar illness, and migraine headaches. It has an acute oral toxicity of III, which means it is slightly harmful if consumed, according to its ADMET characteristics. It can, however, pass the blood-brain barrier. Because valproate is not inhibited by the majority of CYP enzymes, it is less likely to induce drug-drug interactions. It is also not an eye irritant. It can, however, cause hepatotoxicity and is nephrotoxic. It is found in the mitochondrial membrane.

The acute oral toxicity grade of Verbascoside is III, indicating that it is moderately toxic. It lacks the ability to pass the blood-brain barrier. Most CYP enzymes do not inhibit Verbascoside, which is an advantageous thing for drug development. Verbascoside is not eye-irritating, and does not cause hepatotoxicity. It is not harmful to the kidneys. Verbascoside's subcellular location is in the mitochondria. Verbascoside possesses anti-cancer effects and is being studied as a possible treatment for cervical cancer. Its inability to be absorbed by the human intestine, however, may restrict its usefulness.

It can be seen that these compounds had acute oral toxicity as II or III, indicating that they had moderate to low toxicity. Reduced toxicity is a critical component in generating successful drugs. One method for reducing toxicity is to change the chemical structure of the substance, which can diminish or remove harmful effects. Another way of doing so is to change the dosage and mode of administration (MoA) of the drug. Clinical trials to assess the drug's safety and efficacy can be used to identify the effective dose. Toxicity can also be reduced by administering the medicine via non-toxic means such as intravenous or topical administration. Despite their toxicity, these chemicals can still be employed as treatments for cervical cancer by employing these toxicity-reducing techniques.

Following the ADMET experiments, the six compounds were molecular docked using AutoDock 4.2 software. AutoDock is a popular docking tool that predicts ligand binding to a target protein. AutoDock 4.2 was used to dock the ligands with the E7 oncoprotein for this study. A blind docking technique was used in the docking protocol, with the grid centered on the ligand. The AutoDock tool predicts the binding affinity between the ligand and protein using a score function based on multiple energy factors. The docking data were then evaluated using visualization and interaction analysis to determine the major interactions between the ligand and protein. The study's docking results revealed that with E7, all of the

compounds showed a negative binding energy, indicating that the chemicals and the E7 protein had a favorable interaction. Bortezomib and Luteolin were the two compounds with the best docking findings.
It is important to note that before performing docking, E7 protein was energy minimized. This is an important step in protein-ligand docking because it helps to lower the protein's energy, allowing for more accurate and reliable docking results.
The interactions of the best docked compounds with the E7 oncoprotein were investigated further. The presence of hydrogen bonds and hydrophobic interactions between the chemicals and the E7 protein was shown by the interaction analysis.
In the E7 - Bortezomib interaction, there were six hydrophobic interactions mainly with ILE and nine hydrogen bonds (with ASP and GLN). These hydrophobic interactions are critical for the protein-ligand complex's stability. Hydrogen bonds are formed when a hydrogen atom comes into contact with an electronegative atom such as oxygen or nitrogen. Hydrogen bonding are also critical in the protein-ligand complex's stability. The E7 - luteolin interaction revealed one hydrophobic contact and six hydrogen bonds, most of which were with GLY and one with SER. The hydrophobic interaction was identified with the protein's nonpolar region, whereas hydrogen bonds were created between the protein's polar regions and the ligand.
The docking results revealed that all the compounds had a negative binding energy with the E7 oncoprotein. This shows that they have potential as E7 oncoprotein inhibitors and could be researched further as possible drugs for the treatment of cervical cancer.

5. Conclusion

There are more than 15 types of high-risk HPV strains. Out of these, HPV-66 is one of the least explored high-risk HPV types. This in-silico analysis contributes to the understanding of the structure and physicochemical properties of HPV-66/ E7 oncoprotein. The aim of this study was to apply various bioinformatic tools for a better understanding of these oncoproteins for therapeutic application. Analysis of physicochemical properties reveal that the HPV – 66 / E7 protein is unstable in nature.
This research has considerably increased our understanding of cervical cancer development. Because of the role of E7 oncoprotein in the progression of cervical cancer, it is a potential target for the development of novel medications for its therapy.
This study sheds light on the possible use of six chemicals for the treatment of cervical cancer by targeting the E7 oncoprotein, including bortezomib, curcumin, luteolin, sodium butyrate, valproate, and Verbascoside. These compounds' ADMET properties have been examined and evaluated, and while they have oral toxicity, they can still be employed as drugs by lowering their toxicity through various drug design strategies.
In addition, docking experiments showed that all of the compounds, displayed a negative binding energy with E7, with bortezomib and luteolin having the greatest docking results. Further, the hydrogen bonds and hydrophobic interactions between the chemicals and the E7 oncoprotein were identified by the interaction analysis.
The findings of this study are encouraging and suggest that these compounds could be used to treat cervical cancer. However, more research is needed to investigate the efficacy and safety of these drugs in vivo. Additionally, it would be beneficial to investigate the effect of these compounds on other oncoproteins involved in the progression of cervical cancer.

6. References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
2. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020 Jul;40(5):602-608. doi: 10.1080/01443615.2019.1634030. Epub 2019 Sep 10. Erratum in: J Obstet Gynaecol. 2020 May;40(4):590. PMID: 31500479; PMCID: PMC7062568.
3. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis. 2014 Nov;41(11):660-4. doi: 10.1097/OLQ.0000000000000193. PMID: 25299412; PMCID: PMC6745688.
4. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003 Jan;16(1):1-17. doi: 10.1128/CMR.16.1.1-17.2003. PMID: 12525422; PMCID: PMC145302.
5. Juárez-González K, Paredes-Cervantes V, Martínez-Salazar M, Gordillo-Rodríguez S, Vera-Arzave C, Martínez-Meraz M, Sánchez-Suárez P, Vázquez-Vega S. Prevalencia del virus del papiloma humano oncogénico en pacientes con lesión cervical [Prevalence of oncogenic human papillomavirus in patients with cervical lesion]. Rev Med Inst Mex Seguro Soc. 2020 May 18;58(3):243-249. Spanish. doi: 10.24875/RMIMSS.M20000027. PMID: 34002982.
6. Rahmat F, Kuan JY, Hajiman Z, Mohamed Shakrin NNS, Che Roos NA, Mustapa M, Ahmad Zaidi NA, Ahmad A. Human Papillomavirus (HPV) Prevalence and Type Distribution in Urban Areas of Malaysia. Asian Pac J Cancer Prev. 2021 Sep 1;22(9):2969-2976. doi: 10.31557/APJCP.2021.22.9.2969. PMID: 34582669; PMCID: PMC8850893.
7. Lazo PA. The molecular genetics of cervical carcinoma. Br J Cancer. 1999 Aug;80(12):2008-18. doi: 10.1038/sj.bjc.6690635. PMID: 10471054; PMCID: PMC2374277.
8. Dyson N, Howley PM, Münger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science. 1989 Feb 17;243(4893):934-7. doi: 10.1126/science.2537532. PMID: 2537532.
9. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res. 2002 Nov;89(2):213-28. doi: 10.1016/s0168-1702(02)00190-9. PMID: 12445661.
10. Prati B, Marangoni B, Boccardo E. Human papillomavirus and genome instability: from productive infection to cancer. Clinics (Sao Paulo). 2018 Sep 6;73(suppl 1):e539s. doi: 10.6061/clinics/2018/e539s. PMID: 30208168; PMCID: PMC6113919.
11. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009 Dec;30(16):2785-91. doi: 10.1002/jcc.21256. PMID: 19399780; PMCID: PMC2760638.
12. Lin Z, Bazzaro M, Wang MC, Chan KC, Peng S, Roden RB. Combination of proteasome and HDAC inhibitors for uterine cervical cancer treatment. Clin Cancer Res. 2009 Jan 15;15(2):570-7. doi: 10.1158/1078-0432.CCR-08-1813. PMID: 19147762; PMCID: PMC2714480.
13. Kim JE, Lee JI, Jin DH, Lee WJ, Park GB, Kim S, Kim YS, Wu TC, Hur DY, Kim D. Sequential treatment of HPV E6 and E7-expressing TC-1 cells with bortezomib and celecoxib promotes apoptosis through p-p38 MAPK-mediated downregulation of cyclin D1 and CDK2. Oncol Rep. 2014 May;31(5):2429-37. doi: 10.3892/or.2014.3082. Epub 2014 Mar 12. PMID: 24627094

14. Zhao X, Zhang R, Song Z, Yang K, He H, Jin L, Zhang W. Curcumin suppressed the proliferation and apoptosis of HPV-positive cervical cancer cells by directly targeting the E6 protein. Phytother Res. 2023 May 8. doi: 10.1002/ptr.7868. Epub ahead of print. PMID: 37157900
15. Han BR, You BR, Park WH. Valproic acid inhibits the growth of HeLa cervical cancer cells via caspase-dependent apoptosis. Oncol Rep. 2013 Dec;30(6):2999-3005. doi: 10.3892/or.2013.2747. Epub 2013 Sep 20. PMID: 24064712.
16. Feng S, Yang Y, Lv J, Sun L, Liu M. Valproic acid exhibits different cell growth arrest effect in three HPV-positive/negative cervical cancer cells and possibly via inducing Notch1 cleavage and E6 downregulation. Int J Oncol. 2016 Jul;49(1):422-30. doi: 10.3892/ijo.2016.3508. Epub 2016 May 6. PMID: 27176495
17. Wang W, Fang D, Zhang H, Xue J, Wangchuk D, Du J, Jiang L. Sodium Butyrate Selectively Kills Cancer Cells and Inhibits Migration in Colorectal Cancer by Targeting Thioredoxin-1. Onco Targets Ther. 2020 May 27;13:4691-4704. doi: 10.2147/OTT.S235575. PMID: 32547098; PMCID: PMC7263851.
18. Ham S, Kim KH, Kwon TH, Bak Y, Lee DH, Song YS, Park SH, Park YS, Kim MS, Kang JW, Hong JT, Yoon DY. Luteolin induces intrinsic apoptosis via inhibition of E6/E7 oncogenes and activation of extrinsic and intrinsic signaling pathways in HPV-18-associated cells. Oncol Rep. 2014 Jun;31(6):2683-91. doi: 10.3892/or.2014.3157. Epub 2014 Apr 25. PMID: 24789165