ARTICLE

In silico screening suggests dicumarol and demethylwedelolactone as E6-p53 inhibitors in the context of cervical cancer treatment

Honeylaine O. Arcilla[1], Conrad Allan Chong[1], Rod Vincent Borromeo[1], Jessa Louise Cadacio[1], Ranelle Janine L. Asi[1], Jeffrey Clement C. Bungar[1, 2], Javier Lozano-Gerona*[1], and Ahmad Reza F. Mazahery[1]

[1]Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City, Metro Manila 1101 Philippines
[2]Institute of Chemistry, College of Science, University of the Philippines Diliman, Quezon City, Metro Manila 1101 Philippines

ABSTRACT

HPV E6 oncoprotein, together with E6AP can degrade p53, causing genomic instability. This leads to cervical cancer (most prevalent cancer among women worldwide). Many non-flavonoid polyphenols have anti-cancer properties but, despite being a wide family of compounds, remain unexplored as E6-E6AP-p53 binding site inhibitors. We analysed non-flavonoid polyphenols (ZINC15 databank, 285 compounds) and their binding to the site using *in silico* techniques to nominate potential drug candidates. We screened them using Lipinski's rule of five. AutoDock Vina Molecular docking against the binding site suggested dicumarol and demethylwedelolactone (DWL) as potential E6 inhibitors, which have not yet been studied for this purpose, with higher affinity than daphnoretin, a non-flavonoid polyphenol with known inhibitory properties against E6. Target Prediction by ChEMBL revealed that both compounds potentially interact with the cancer-marking genes ROS1 and NQO1. ADMET profile suggests both compounds may be developed into an oral drug.

*Corresponding author
Email Address: jlozano1@up.edu.ph
Date received: 19 June 2025
Dates revised: 08 September 2025, 02 October 2025
Date accepted: 28 October 2025
DOI: https://doi.org/10.54645/202518SupKQU-82

INTRODUCTION

Cervical cancer is the fourth most common cancer in women and the fourth leading cause of cancer death worldwide. In 2020, 604,000 new cases and 342,000 deaths related to cervical cancer were approximated (Sung et al., 2021). This cancer is known to be caused by the human papillomavirus (HPV), a sexually transmitted virus highly prevalent in the population. Globally, DNA from high-risk HPV strains is found in 79% to 100% of invasive cervical cancer cases (de Sanjose et al., 2010). For this reason, research priority that would lead to better therapeutic approaches towards this type of cancer is necessary.

The HPV vaccines that are currently available are only prophylactic and have no therapeutic impact against an ongoing infection (Hancock et al., 2018). Due to cost, a lack of reproductive health platforms for adolescents, cultural barriers, and difficulties reaching the target population, many women in

KEYWORDS

cervical cancer, HPV16 E6 oncoprotein, p53, molecular docking, nonflavonoid polyphenols, Autodock Vina, Cancer, natural products, HPV, in silico

low to middle-income countries do not have access to conventional screening and prevention approaches, which results in a disproportionately high burden of cervical cancer associated with late-stage diagnosis (Denny, 2015). Furthermore, current treatments for cervical cancer, such as surgery, radiation, and chemotherapy, are associated with significant morbidity and often result in decreased quality of life. (Chakraborty & Rahman, 2012). Cervical cancer is costly and challenging to treat and would benefit from the development of alternative drugs (Gupta et al., 2022). In particular, natural products have been a very promising source of affordable adjunct therapies against many diseases including cancer.

Polyphenols are a large group of natural plant-derived chemicals providing preventive and therapeutic potential against cancer (Zhou et al., 2016). Polyphenols are classified into five groups, namely phenolic acid, flavonoid, coumarins, stilbenes, and lignans (Moga et al., 2016). While flavonoids are the most well-known and extensively researched group of polyphenols, there are numerous non-flavonoid polyphenols, which constitute a significant proportion of the polyphenolic content in many plant species, that have received less attention. Despite being understudied, evidence suggests that non-flavonoid polyphenols have shown promise as potential cervical cancer therapeutics in experimental studies (Baghdadi et al., 2018; Chatterjee et al., 2018; Park, 2017). Their full anti-cancer potential however is not fully realized due to the tedious work needed for bioassay guided purification of natural products.

In recent years, various simulation methods have allowed researchers to screen for potential candidate molecules that could be isolated from natural products. For instance, Molecular docking, a structure-based *in silico* approach, can screen an extensive library of compounds with promising properties. It predicts a compound's binding affinity and conformation toward the target and visualises the ligand-target interactions (Gomes et al., 2021). The data obtained can then be applied to *in vitro* research for verification. *In silico* approaches enable the simultaneous screening of a large number of compounds, saving valuable time and laboratory resources by narrowing down which one to focus on for *in vitro* testing. This is of particular interest in the context of studying compounds found in the rich flora from the South East Asia region, as many have folkloric usage but their possible mechanisms of action are relatively unexplored. Particularly, how these compounds would act against specific cancer biomarkers need to be elucidated.

The E6 oncoprotein has been a biomarker of interest for cervical cancer. It is constitutively expressed in cervical cancer cells and is essential for the formation and advancement of this type of cancer (Pal & Kundu, 2020). Its binding residues are highly conserved in high-risk HPV strains such as HPV16, which cause about 70% of cervical cancers (Martinez-Zapien et al., 2016; de Sanjose et al., 2010). Martinez-Zapien et al (2016) published crystallization data (PDB code 4xr8) demonstrate that E6 binds to the ubiquitin ligase E6AP (E6-associated protein) and forms the E6-E6AP-p53 complex, which causes the ubiquitination and degradation of p53, bypassing normal regulation. The cell subsequently develops genomic instability, and ultimately develops cancer (Li et al., 2019).

p53 degradation by E6 depends critically on the conformational integrity of this p53 binding domain (Bernard et al., 2011). The majority of research has focused on the E6-E6AP interaction (Cherry et al., 2013; Kumar et al., 2019; Prakash et al., 2020; Zanier et al., 2014), with only a few studies looking into potential E6-p53 inhibitors (Celegato et al., 2020; Nabati et al., 2020). However, these studies use a different mode of assembly of the E6 structure because they only use the one that was previously determined from the E6/p53 dimeric complex. It was later that the structure of E6 as part of the trimeric complex E6/E6AP/p53 was identified (Li et al., 2019). We focused on screening ligands that can disrupt the protein–protein interaction between E6 and p53. Specifically, we targeted the E6 binding surface, which is highly conserved and critical for complex formation. By using the E6 structure in its bound conformation, we ensured that the docking simulations captured the relevant interaction interface. This approach may help identify ligands capable of preventing E6-mediated p53 degradation during HPV-related carcinogenesis.

In the present study, we used *in silico* analysis to determine which non-flavonoid polyphenol compounds have high binding affinities to the E6-p53 binding site using the E6 protein structure isolated from the ternary complex E6/E6AP/p53 as template, to be proposed as a candidate disruptors. We hope these results may streamline further research into the matter by suggesting which compounds ought to be prioritized.

MATERIALS AND METHOD

Protein Preparation

We retrieved the high-resolution 3D structure of the HPV16 E6/E6AP/p53 ternary complex (PDB Code: 4xr8) in PDB format from the Protein Data Bank (PDB) (https://www.rcsb.org/) (Berman et al., 2000). To reduce the computational requirements, the F-chain was chosen between the two superimposable chains of E6 oncoprotein from the duplicate crystal E6/E6AP/p53 core heterotrimers when performing the Autodock VINA analysis. This is a limitation in our analysis, but we do not expect important changes from the 2 possible structures as the variations do not interact with E6AP nor p53 (Martinez-Zapien et. al., 2016).

As established in the study of de Sanjose et. al, 2010, since the high-risk strain HPV-16 is associated with 70% of invasive cervical cancer, we solely focused on this and other protein variations were not considered in this analysis. The protein file was prepared in AutoDock MGL Tools v1.5.7 (https://ccsb.scripps.edu/mgltools/) (Morris et al., 2009) to set the proper condition of the 3D structure for docking.

During preparation, molecules such as the E6AP, p53, and other small molecules in the crystal structure complex were removed, leaving a single chain of E6 oncoprotein, which AutoDock Vina can analyze. Protein preparation also included the removal of all water molecules, required by the technique (Wong & Lightstone, 2011). AutoDock MGL Tools automatically adds Kollman charges via a built-in setting to set the proper condition of the 3D structure for docking. This step takes into account electrostatic interactions between proteins and ligands and prevents van der Waals interactions from dominating.

Of note, this step eliminates the direct effect of E6AP and p53 with the molecules tested, but does retain the structure in which the E6 is found when forming the ternary complex. We estimate that this is the structure that the potential inhibitors would find the E6 to be in. This also indicates that our study merely suggests compounds that have the potential to interact initially with E6 when it forms part of the ternary complex, likely reducing the complex stability. But it does not assure that the disruption through competitive inhibition of the p53 binding site may be truly effective or remain long term. Further studies exploring the molecular dynamics of the interaction are suggested for future follow-up studies, here we are proposing potential candidates for it.

Ligand Preparation

We first prepared a ligand database of nonflavonoid polyphenols from the publications of Abbaszadeh et al., (2019) and Abotaleb et al., (2020). Then we checked the availability of their 3D structures in the ZINC15 database, developed by Sterling and Irvin (2015), and the appropriate candidate isomers of the ligands to be analyzed were selected (https://zinc15.docking.org/). ZINC15 is preferred because of its user-friendliness even for non-specialists and most of all, compared to earlier versions, the molecules are all biologically relevant and include the 3D structure.

We used Lipinski's Rule of Five (MW< 500Da, H-bond donors < 5, H-bond acceptors < 10, and logP< 5) as a limiting criterion to filter the ligands with unsuitable pharmacokinetic properties. This was done by manually inputting the SMILES notation found on the ZINC15 profile of each compound into Molinspiration (https://www.molinspiration.com/) (Molinspiration, n.d.).

The ligands were prepared using the OpenBabel v3.1.1 (O'Boyle et al., 2011) command line to convert SDF ligand files into PDBQT file format.

Molecular Docking

The program AutoDock vina , developed by Trott & Olson (2010) was selected as the main program for molecular docking simulations. We aimed to rank the nonflavonoid polyphenols based on their binding affinity to the E6-p53 binding site and identify the interacting amino acid residues. This program was preferred over the classic AutoDock program since it allowed faster screening of multiple ligands. Martinez-Zapien et al.(2016) specified the amino acid residues Gln6, Glu7, Arg8, Arg10, Gln14, Glu18, Tyr43, Asp44, Phe47, Asp49, Leu100, and Pro112 for the specific E6-p53 binding site which was used during docking.

Daphnoretin was used as a positive control to compare the docking results. This compound has been predicted in a similar study to be the most potent E6 inhibitor amongst several natural products screened with a binding affinity of -8.3 Kcal/mol (Mamgain et. al, 2015). Only the ligands with better or equal binding affinity than daphnoretin were considered.

Re-validation of the identified top-ranking ligands was performed using the BIO-HPC Achilles Blind Docking Server (https://bio-hpc.ucam.edu/achilles/), which uses an internal version of MetaScreener (https://github.com/bio-hpc/metascreener). The binding scores were compared to Autodock Vina.

Post-docking Visualization and Analysis

To analyze the ligand-protein interaction and visualize the resulting protein-ligand complex, we used the Protein-Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) to generate 3D representations (Adasme et al., 2021) and LigPlot+ (Laskowski & Swindells, 2011) for 2D diagrams.

We also calculated the predicted inhibition constants (pKi) using the formula pKi = −log10 Ki where Ki is the equilibrium constant, derived as shown in Eq 1.

Equation 1:

$\Delta G = RTlnK_i \Rightarrow K_i = e^{\Delta G/RT}$; where ΔG is the Gibbs free energy, R is the ideal gas constant (1.98719 cal·mol-1·K-1), and T is the temperature (assumed at 298.15 K).

Although our study does not definitely confirm the nature of the inhibition, from the fact that the interaction is at the E6-p53 binding site, here we assume that the compounds may likely act through competitive inhibition. Thus, we estimated that half-maximal inhibitory concentration (pIC50) may be approximated as Ki = IC50/2 when [S] (substrate concentration) = Km (Michaelis-Menten constant) (Haupt et al., 2015). Nevertheless, this ought to be confirmed in follow-up studies. We use this simplification here for the purposes of ranking potential compounds and make the final selection of potential druggable substances.

Target Protein Prediction and Pathway Analysis

We used the ChEMBL database (https://www.ebi.ac.uk/chembl/) to predict which other proteins the highest-ranking ligands would bind to (Mendez et al., 2019). From the two levels of confidence CHEMBL offers (70-90%), we only included "active" compounds predicted to interact with the target with a 90% confidence level (Bosc et al., 2019).

ADME/T Analysis

The ADME/T (absorption, distribution, metabolism, excretion, and toxicity) parameters of the selected ligands, as well as daphnoretin, the positive control. This evaluation was done by inputting the SMILES format of the screened ligands into the admetSAR 2.0 web tool (http://lmmd.ecust.edu.cn/admetsar2/) (Yang et al., 2019). In addition, Doxorubicin was included as an additional reference. Doxorubicin is not known to interact with E6, but it was included in the ADME/T analysis since it is usually used as a first-line drug for cervical cancer treatment (Johnson-Arbor K. & Dubey R. 2023) that induces apoptosis in cancerous cells. ADMET properties ensure the safety and efficacy of these compounds, giving them a higher chance of becoming drug candidates in future clinical trials.

RESULTS

As shown in Figure 1, we compiled a list of 481 nonflavonoid polyphenols (Abbaszadeh et al., 2019; Abotaleb et al., 2020). From this list, we identified 333 compounds whose 3D structures are available in the ZINC15 database (Sterling & Irwin, 2015). From those, we identified 285 compounds that were pharmacokinetically suitable after applying Lipinski's Rule of Five. These 285 compounds were subjected to molecular docking analysis in AutoDock Vina, and their binding affinity to the E6-p53 binding site was compared to that of the positive control, daphnoretin. Based on the docking results, we chose the top 14 compounds which had better or comparable binding affinity to the E6-p53 binding site than daphnoretin.

The top 14 compounds were subjected to post-docking visualization and analysis. To our knowledge, only two of these compounds, dicumarol and DWL, had not yet been studied in cervical cancer research. We further subjected these two compounds to target prediction, pathway analysis, and ADMET analysis. As a result, we identified dicumarol and DWL to have promising anti-cervical cancer and drug-like properties, which should be validated through *in vitro* experiments. Figure 1 depicts a schematic diagram of the research pipeline.

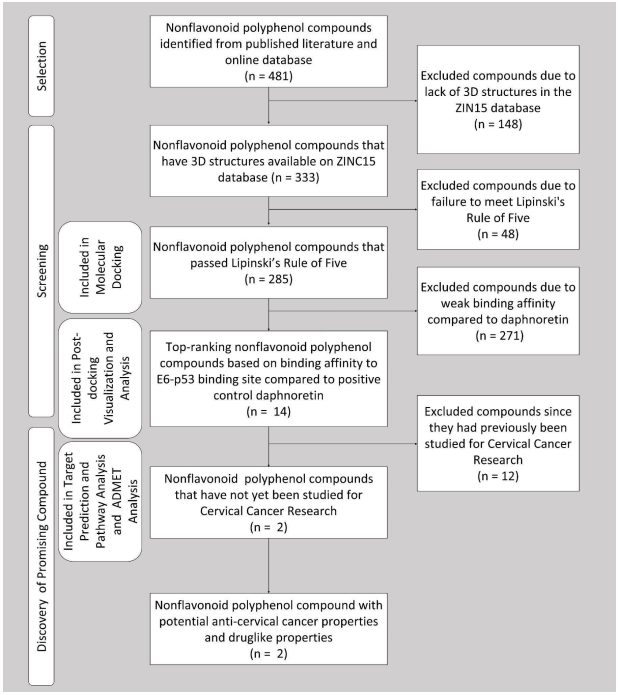


Figure 1: Research Pipeline. Schematic depiction of the steps performed; selection, screening, and discovery of promising compounds. The different in silico analyses conducted, the number of remaining compounds per stage based on a specific criterion, and the number of compounds excluded are also highlighted.

Molecular docking shows 14 nonflavonoids with similar or better binding affinity than daphnoretin for HPV16 E6.

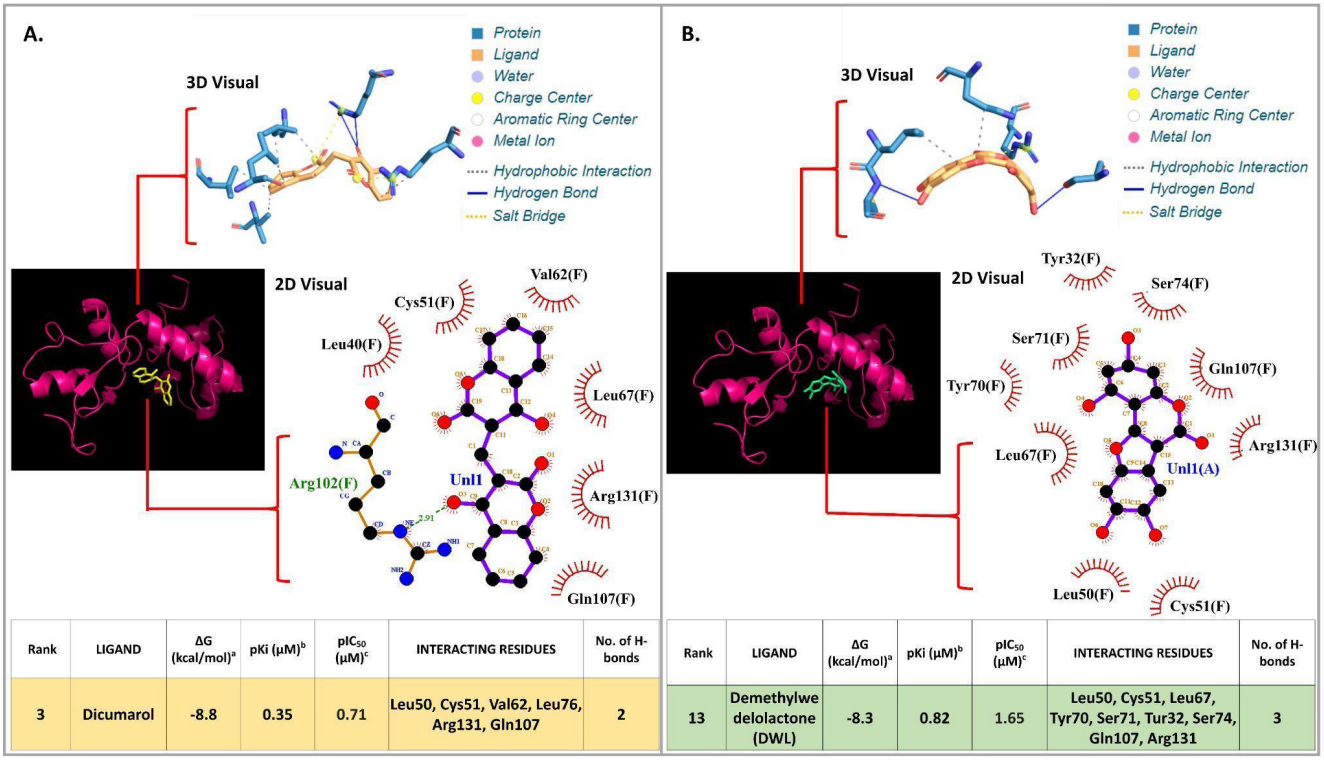
The results of molecular docking in AutoDock Vina showed that 14 nonflavonoid polyphenol compounds bind to the E6-p53 binding site with comparable or higher affinity than the positive control, daphnoretin. The binding affinities of these compounds range from -9.1 kcal/mol to -8.3 kcal/mol. These compounds, ranked from highest to lowest binding affinity, are picropolygamain (analog 1), gerberinol, dicumarol, calanolide A (analog 1), 7-hydroxyenterolactone, calanolide A (analog 2), psoralidin, felamidin, picropolygamain (analog 2), llagate, 5-methoxyhinokinin, ammoresinol, demethylwedelolactone (DWL), and fraxine (analog 1) (Table 1).

In order to predict whether the E6-p53 binding site would be the location preferred for docking, we analysed the compounds using the Achilles Blind Docking program. The results (shown in table 1). Of note, dicumarol provided the same binding energy for the docking, indicating similar docking site and fit; while DWL had a lower energy, likely from a different binding site. Therefore, DWL may have an alternative binding site whose effect we cannot predict (Table 1).

Table 1: Comparison of binding affinities obtained from Autodock VINA and Achilles

LIGAND	Autodock VINA (kcal/mol)	Achilles (kcal/mol)
Picropolygamain (Analog 1)	-9.1	-9.2
Gerberinol	-8.9	-8.9
Dicumarol	-8.8	-8.8
Calanolide A (Analog 1)	-8.7	-8.7
7-Hydroxyenterolactone	-8.6	-8.6
Calanolide A (Analog 2)	-8.6	-8.9
Psoralidin	-8.6	-8.7
Felamidin	-8.5	-8.5
Picropolygamain (Analog 2)	-8.5	-8.2
Llagate	-8.4	-8.5
5-Methoxyhinokinin	-8.4	-8.1
Ammoresinol	-8.3	-8.3
Demethylwedelolactone (DWL)	-8.3	-7.1
Fraxine (Analog 1)	-8.3	-8.0
Daphnoretin	-8.3	-8.2

From the expected binding energies (ΔG), we also calculated the predicted inhibition constants (pKi) and estimated half-maximal inhibitory concentrations (pIC50). This is shown in Supplementary Table 1. Additional information, such as interacting amino acid residues and the number of hydrogen bonds is also shown in Figure 2.



Rank	LIGAND	ΔG (kcal/mol)[a]	pKi (μM)[b]	pIC_{50} (μM)[c]	INTERACTING RESIDUES	No. of H-bonds
3	Dicumarol	-8.8	0.35	0.71	Leu50, Cys51, Val62, Leu76, Arg131, Gln107	2

Rank	LIGAND	ΔG (kcal/mol)[a]	pKi (μM)[b]	pIC_{50} (μM)[c]	INTERACTING RESIDUES	No. of H-bonds
13	Demethylwedelolactone (DWL)	-8.3	0.82	1.65	Leu50, Cys51, Leu67, Tyr70, Ser71, Tur32, Ser74, Gln107, Arg131	3

Figure 2: 2D and 3D visualizations of Ligand-HPV16 E6 complexes. Interactions between the (A) dicumarol and (B) DWL and the E6-p53 binding site after molecular docking. The figure on the left portion visualizes the 3D structure of the protein (ribbon) with the predicted top binding mode of the docked ligand (left - dicumarol, right - DMW). The upper right figure depicts the 3D structure, while the lower right depicts the 2D structure of each ligand-protein complex. The rank, name, binding energy (G), calculated predicted inhibition constants (pKi) values, estimated half-maximal inhibitory concentrations (pIC50), and interaction data, such as interacting amino acid residues and the number of hydrogen bonds, are all listed in the table below.

Protein and pathway analysis predicts ROS1 and NQO1 genes to interact with and may reinforce the effect of dicumarol and demethylwedelolactone.

Table 2 displays the active genes of target proteins common to dicumarol and DWL (ROS1, and NQO1), for dicumarol only (MAP2, CHRNB4, and HSD17B3), and for DWL only (HTR3A, ADORA2B, CAPN1, and KDM1A), with a 90% confidence level based on ChemBL target prediction.

Table 2: Active genes of target proteins common between dicumarol and DWL, for dicumarol only, and for DWL only at 90% confidence based on ChemBL

	Abbreviation	Gene name
Common between dicumarol and DWL	*ROS1*	Proto-oncogene tyrosine-protein kinase ROS
	NQO1	NAD(P)H:quinone acceptor oxidoreductases (NQO1)
Dicumarol only	*MAP2*	MAP2 microtubule-associated protein 2
	CHRNB4	Cholinergic receptor nicotinic beta 4 subunit
	HSD17B3	hydroxysteroid 17-beta dehydrogenase 3
DWL only	*HTR3A*	5-hydroxytryptamine receptor 3A
	ADORA2B/ A2BAR	adenosine A2b receptor
	CAPN1	calpain 1
	KDM1A/LSD	lysine-specific histone demethylase 1

The number of active genes found (in Table 2) was too small to proceed with a reliable pathway analysis (PANTHER, nor GeneMANIA).

Furthermore, from the list of 14 compounds, we investigated those that were found to have an effect on cervical cancer in previous studies. We have discovered that ROS1 is an active gene target of picropolygamain via ChemBL, which has inhibitory effects on cervical cancer (Gigliarelli et. al., 2018).

This finding reinforces the notion that ROS1 may be a viable target for cervical cancer. Two other compounds from Table 1, felamidin and fraxine, target ROS1 and should be tested on potential anticancer effect.

ADMET Profiles of dicumarol and DWL exhibit potential druglike properties

ADMET analysis showed that dicumarol and DWL exhibit potential drug-like properties in treating cervical cancer, which can be validated further using *in vitro* studies. The main predicted ADMET properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity) are listed in Table 3. The totality of ADMET results can be seen in Supplementary Table 2.

The admetSAR 2.0 tool uses a scale of 0 to 1 to predict the result of the analysis, which is expressed as either a positive or negative result. The probability value assigned to a specific result reflects the accuracy of the predictions. Over 0.700 probability of the result indicates a high probability, thus greater prediction reliability (Dulsat et al., 2023).

The ADMET values suggest that Dicumarol and DWL may have a better bioavailability than the control daphnoretin or the reference chemotherapy drug doxorubicin. As they are positive for Human Intestinal Absorption (HIA), and dicumarol is also positive for Human Oral Bioavailability (HOB). Nevertheless, these values must be confirmed in future studies as the compounds were also Caco2 negative, another predictor of intestinal absorption.

Overall the results predict that dicumarol is likely more easily absorbed than the reference compounds. While DWL is possibly more easily absorbed than the reference drug doxorubicin, but not necessarily than the compound daphnoretin.

Regarding the P-glycoprotein inhibitor parameter, both compounds, dicumarol and DWL, were predicted to have a high probability of being non-inhibitors and non-substrates of P-glycoprotein.

Blood-brain barrier (BBB) permeability is critical in determining its efficacy in reaching the brain. Both compounds, similar to daphnoretin and doxorubicin, demonstrated a high likelihood of being poorly absorbed in the brain.

Table 3: ADMET Predicted Profile of Dicumarol and DWL generated using admetSAR 2.0. Green colored cells indicate positive results or low risk, while red indicates negative results or high risk. The absence of color indicates that the prediction is inconclusive

Parameter	Dicumarol		DWL		Daphnoretin		Doxorubicin	
	Result	Probability	Result	Probability	Result	Probability	Result	Probability
ABSORPTION								
Human Intestinal Absorption	HIA+	0.9125	HIA+	0.8631	HIA+	0.9493	HIA-	0.6934
Human Oral Bioavailability	HOB+	0.800	HOB-	0.5429	HOB+	0.5429	HOB-	0.9143
$Caco_2$ Permeability	$Caco_2$-	0.7577	$Caco_2$-	0.7614	$Caco_2$-	0.5519	$Caco_2$-	0.8650
Blood Brain Barrier	BBB-	0.9250	BBB-	0.775	BBB-	0.8250	BBB-	0.9750
P-glycoprotein inhibitor	Non-inhibitor	0.8996	Non-inhibitor	0.9015	Inhibitor	0.7126	Non-inhibitor	0.9166
P-glycoprotein substrate	Non-substrate	0.9647	Non-substrate	0.9035	Non-substrate	0.8821	Substrate	0.9478
DISTRIBUTION								
Subcellular Localization	Mitochondria	0.8402	Mitochondria	0.6365	Mitochondria	0.7332	Nucleus	0.8460
METABOLISM								
CYP1A2 inhibition	Non-inhibitor	0.7905	Inhibitor	0.7113	Inhibitor	0.7468	Non-inhibitor	0.9045
CYP2C19 inhibition	Non-inhibitor	0.6071	Non-inhibitor	0.7590	Non-inhibitor	0.9025	Non-inhibitor	0.9025
CYP2C9 inhibition	Inhibitor	0.8948	Non-inhibitor	0.8152	Non-inhibitor	0.9071	Non-inhibitor	0.9209
CYP2C9 substrate	Substrate	1.000	Non-substrate	0.8053	Non-substrate	0.8283	Non-substrate	1
CYP2D6 inhibition	Non-inhibitor	0.9681	Non-inhibitor	0.8619	Non-inhibitor	0.9305	Non-inhibitor	0.9231
CYP2D6 substrate	Non-substrate	0.8559	Non-substrate	0.8053	Non-substrate	0.8191	Non-substrate	0.8188
CYP3A4 inhibition	Non-inhibitor	0.9098	Inhibitor	0.6142	Non-inhibitor	0.8440	Non-inhibitor	0.831
CYP3A4 substrate	Non-substrate	0.5787	Non-substrate	0.5608	Non-substrate	0.5000	Substrate	0.7321
CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	0.9165	Low CYP inhibitory promiscuity	0.6873	Low CYP inhibitory promiscuity	0.8566	Low CYP inhibitory promiscuity	0.8911
EXCRETION AND TOXICITY								
Carcinogenicity (binary)	Non-carcinogens	0.9613	Non-carcinogens	1.000	Non-carcinogens	0.9900	Non-carcinogens	0.9300
Carcinogenicity (trinary)	Non-required	0.6978	Non-required	0.5756	Non-required	0.4852	Non-required	0.6246
Ames mutagenesis	Non-AMES toxic	0.6300	Non-AMES toxic	0.7300	Non-AMES toxic	0.6400	AMES toxic	0.9900
Human either-a-go-go inhibition	Non- hERG inhibitor	0.7708	Non- hERG inhibitor	0.9075	Non- hERG inhibitor	0.6615	Non- hERG inhibitor	0.5316
Acute Oral Toxicity (c)	II	0.7149	II	0.5429	III	0.6713	III	0.7766

The distribution category data revealed that dicumarol is highly likely to be concentrated in the mitochondria, similar to daphnoretin. However, DWL has a low probability of being present in this organelle while doxorubicin showed a high probability of concentration in the nucleus.

Aside from its action in p53 degradation, HPV16 E6 overexpression causes mitochondrial damage and oxidative stress, leading to DNA damage in cancer cervical cells (Evans et al., 2016), while dicumarol was found to induce cytotoxicity in human pancreatic cancer cells by producing reactive oxygen species and oxidative stress, leading to cell death (Du et al., 2006). Thus, the predicted distribution of dicumarol in the mitochondria suggests its potential to target mitochondrial pathways for other anticancer effects, making it a possible treatment for cervical cancer cells.

The data for metabolism showed predicted values of a compound's interaction with various cytochrome P450 enzymes. The low inhibitory promiscuity of dicumarol and DWL implies that they are less likely to interact with other drugs and are predicted to have a low probability of inhibiting most CYP isoforms. Therefore, there is a lower chance of experiencing adverse effects or drug-drug interactions associated with CYP metabolism (Cheng et al., 2011).

In terms of excretion and toxicity, the results of the analysis are as follows: for Carcinogenicity (binary), the data revealed that both compounds, similar to daphnoretin and doxorubicin, are likely non-carcinogenic, whereas, for Carcinogenicity (trinary), the predicted carcinogenicity requirement for the DWL and dicumarol is non-required. The binary carcinogenicity model classifies a substance as either carcinogenic or non-carcinogenic. The trinary classification system predicts the carcinogenic potential of a compound by classifying it as either non-required, warning, or dangerous. These categories are determined by evaluating the median toxic dose (TD50) or the minimum dosage level necessary to produce a toxic effect in 50% of the population (Cheng et al., 2012).

The Ames mutagenicity test was used to assess potential teratogenicity and genotoxicity (Guan et al., 2018). Both compounds are predicted to be non-AMES toxic. Similar to daphnoretin, dicumarol may have a low likelihood for this parameter, while DWL had a higher probability of being non-toxic than these two. At the same time, doxorubicin was found to be highly likely to be AMES toxic. Both dicumarol and DWL showed a high probability of not inhibiting hERG, which gives them an advantage over daphnoretin and doxorubicin, as the latter compounds have some probability of hERG inhibition. Regarding Acute Oral Toxicity (c), the analysis predicted that the dicumarol and DWL would be classified as toxicity class II ($5\ mg/kg < LD50 \leq 50\ mg/kg$). Worse than daphnoretin and doxorubicin, predicted to be class III ($50\ mg/kg < LD50 \leq 300\ mg/kg$). Due to higher absorbance, it is possible that lesser concentrations may still reach their target; this critical aspect must be studied through *in vitro* and *in vivo* follow up studies.

DISCUSSION

Through molecular docking, we found 14 nonflavonoid polyphenols with better or comparable binding affinity to the E6-p53 binding site than the drug daphnoretin. To tackle the large-scale selection, we applied the Lipinski's Rule of Five as a limiting criterion. These five drug-like characteristics (Li et al, 2017) select for appropriate molecular weight of less than 500 Da (Matsson & Kihlberg, 2017); lipophilicity of logP lesser than 5, to cross lipid membranes (Liu et al., 2009); appropriate hydrogen bond donor (no more than 5) and acceptor (no more than 10) availability (Chen et al., 2016; Coimbra et al., 2020). This excludes most compounds with poor pharmacokinetic properties (Yang & Hinner, 2015).

Only dicumarol and demethylwedelolactone (DWL) were identified as having not yet been studied in cervical cancer. Dicumarol is a naturally occurring hydroxycoumarin isolated from rotting *Melilotus officinalis* (L.) Pallas as a rodenticide (Sun et al., 2020). DWL, on the other hand, is a naturally occurring coumarin discovered in *Eclipta alba* (Lee et al., 2012; Syed et al., 2003). Both dicumarol (Asher et al., 2002; Buranrat et al., 2010; Du et al., 2006; Matsui et al., 2010; Watanabe et al., 2006) and DW (Lee et al., 2012) have previously demonstrated anticancer activity in other cancer types, including myeloid leukemia, liver cancer, breast cancer, urothelial cancer, urogenital cancer, and lung cancer cells.

Dicumarol and DWL were predicted to be potential inhibitors of the E6-p53 binding site based on the calculated pharmacodynamic parameters and interaction data (see Figure 2). The expected Ki for both compounds is lesser than the IC50 of the substrate, thus they are expected to provide competitive inhibition (Burlingham & Widlanski, 2003). Importantly, dicumarol forms two H-bonds (Arg102) and DWL three (one Cys51, two Ser74). These interactions are suspected of driving the potential inhibitory effect (Kumar et al., 2019) because of their stability (Bulusu & Desiraju, 2020). In addition, the residues that make a key hydrophobic pocket of E6 (Leu50, Cys51, Val62, Leu67, Arg 102, Gln107, and Arg131) (Kolluru et al., 2019) are occupied by interactions for both dicumarol and DWL. The other top 14 binding compounds did not interact with the amino acid residues indicated in the E6 part of the structure to be in direct contact with p53. Suggesting the existence of novel interactions between compounds and E6, which may or may not disrupt the oncoprotein's interaction with p53, this detail may require to be investigated further using molecular dynamics simulation and *in vitro* assays. Dicumarol and DWL interact with amino acids adjacent to those that interact with daphnoretin, our positive control. Leading us to infer that daphnoretin occupies a similar space in the binding pocket of E6 as dicumarol and DWL.

Of the two, dicumarol was predicted to have a higher binding affinity, as well as provide the same result in both guided and blind docking analysis. Therefore, we estimate that its inhibitory effect is likely higher than DWL.

Target prediction analysis in ChemBL showed that dicumarol and DWL have additional target proteins involved in cancer-related pathways. Both compounds were predicted to inhibit *ROS1* and *NQO1*. *ROS1* is a member of the sevenless subfamily of tyrosine kinase insulin receptor genes, known to regulate growth, although its physiological role is unclear (D'Angelo et al., 2020). It is most commonly associated with non-small cell lung cancer (Drilon et al., 2021; Klempner & Ou, 2015), but also it is expressed to a lesser extent in cervix and colon cancer. NQO1, a NAD(P)H dehydrogenase (quinone) family member, is a multifunctional antioxidant enzyme with higher expression levels in many solid tumors than in surrounding normal tissue. It is thought to protect cancer cells from anticancer drugs by detoxifying intracellular oxidative stress (Matsui et al., 2010).
The genomic changes of *ROS1* often lead to gene fusion with numerous fusion partners, all of which are significant oncogenic drivers. *ROS1* kinase activity is constitutively activated, resulting in increased cell proliferation, survival, and migration via the PI3K/AKT, JAK/STAT, and MAPK/ERK signaling pathways (Roskoski, 2017). On the other hand, studies on the biological significance of *NQO1* in cancer have proven contradictory. To inhibit tumor growth caused by carcinogens, *NQO1* is activated with defensive genes that protect against

various stresses. However, *NQO1* has also been linked to carcinogenesis by reductively activating environmental carcinogens (Ma et al., 2014; Matsui et al., 2010).

In the cervix, according to Ma et al., (2014), aberrant *NQO1* expression renders it more susceptible to HPV infection; as a result, HPV infection may accelerate *NQO1* overexpression and enhance cervical squamous cell carcinoma invasion and metastasis. The activation of the *NQO1* gene in human colon adenocarcinoma and hepatoma cells by hypoxia and mitomycin C plays a role in the NF-kB signaling pathway, which is important for cervical cancer cell proliferation, resistance to apoptosis, invasion, and metastasis (Ma et al., 2014).

To our knowledge, no research findings relating *ROS1* or *NQO1* to the HPV16 E6 oncoprotein have been published, but the research mentioned in other tissues suggests a potential benefit to this additional predicted effect of dicumarol and DWL. We believe that the two compounds may provide an additional antitumoral effect for cervical cancer in particular thanks to their interaction with these two proteins. These findings in our simulatory experiments however will need validation ideally through an *in vitro* experiment.

It should be noted that possible synergistic effects of dicumarol and DWL were not explored in this study. Although some anticancer molecules can work better in combinatorial treatment with other similar molecules, we presuppose that under the conditions of our simulation, there might be possible competitive binding between the two molecules on E6-P53, with no synergy possible. We recommend a detailed investigation of their individual effects in an *in vitro* experiment be done first.

Dicumarol and DWL were evaluated for ADMET properties (Table 3). Both Dicumarol and DWL showed a high probability of having high absorption for HIA, indicating that these compounds have the potential to be absorbed into the bloodstream from the small intestine. In addition, Dicumarol was found to have a high probability of high absorption for HOB which indicates that it has the potential to be absorbed straight from the gastrointestinal tract into the bloodstream after oral administration, unlike DWL. However, both candidate compounds showed low absorption for $Caco_2$, the main gastrointestinal absorption mechanism. We conclude that we can expect some degree of oral bioavailability for both compounds, particularly for Dicumarol, but this must first be confirmed with *in vivo* assays.

Importantly, both drugs are predicted to not cross the blood-brain barrier (BBB). A low brain permeability predicts fewer off-target and side effects (Alvarez et al., 2021). This would not reduce their usefulness in chemotherapy for cervical cancer substantially, as the incidence of metastasis to the brain is relatively low for this type of cancer (Divine et al., 2016). Both compounds were discovered to be P-gp noninhibitors and nonsubstrates. Drugs that are substrates of P-gp are actively transported out of cells by P-gp-mediated drug transport, which hinders therapy efficacy (Amin, 2013). Thus, we expect Dicumarol and DWL to have good bioavailability and not interfere with that of other drugs that may interact with P-gp.

Metabolism is a predicted category that indicates whether a drug interacts with CYP450 enzymes as a substrate or inhibitor. Only Dicumarol showed potential reactivity, which we confirmed using the online tool Biotransformer 3.0 (Wishart et. al. 2022). It is important to note, however, that these tools are not ideal to determine the overall drug stability (Dulsat et al., 2023) and may need to be confirmed.

The binary carcinogenicity model indicates that both compounds are non-carcinogenic and have been classified as non-required under the trinary classification system, indicating that they are not expected to cause cancer. Both compounds were predicted to be non-mutagenic and potentially less harmful to human use or consumption compared to doxorubicin, currently in clinical use.

Moreover, both compounds were predicted to be non-hERG-inhibitors, which means they may not inhibit the hERG potassium channel, which is vital in the cardiac action potential. Inhibiting the hERG potassium channel is a major problem in the early stages of drug development because it causes QT interval prolongation and severe cardiac side effects (Wang et al., 2012). The oral toxicity of dicumarol and DWL falls between the values of our controls Daphnoretin and Doxorubicin (See Supplemental Table 2), and fall under category 2 ($5\ mg/kg < LD50 \leq 50\ mg/kg$), meaning that they may pose a moderate to high risk to human health (Guan et al., 2018). Despite this, dicumarol and DWL may still have therapeutic potential because overall the analysis predicted that both compounds had a favorable safety profile. This important detail must be confirmed through *in vitro* and *in vivo* follow up studies. Overall, the prediction suggests that dicumarol and DWL may have potential druglike qualities that could provide a novel alternate treatment option for cervical cancer.

Although dicumarol and DWL are understudied, both are commercially available and can be obtained from suppliers in their purified form. As a result, the time-consuming process of extracting and isolating these compounds from their raw materials is no longer necessary. Therefore, dicumarol and DWL may be potential candidates for further testing to determine their effect on cervical cancer cells. We suggest a confirmatory *in silico* study to determine molecular dynamic characteristics of the interaction, both with the ternary structure E6/E6AP/p53, and without the p53 participation to confirm the long term stability of their inhibition. Should this study support their effectiveness, this ought to be followed up by *in vitro* testing to determine their direct interaction with the viral proteins as well as possible role on the expression of *ROS1* and *NQO1*, and finally the effect on cervical cancer cells.

LIMITATIONS OF THE STUDY

In this study we used the structure of the E6 protein as found in the ternary structure of E6/E6AP/p53. But only the E6AP and p53 proteins were ignored for easier computation. Thus the possible direct interaction of these 2 proteins on the ligands is not accounted for. In addition, the long term stability of the proposed ligands is limited by the absence of molecular dynamics (MD) simulations and MM-GBSA rescoring analyses, which could provide deeper insights into the stability and energetics of ligand–protein interactions. Consequently, the docking results presented here should be interpreted with caution and viewed primarily as hypothesis-generating. Future work incorporating MD simulations and free-energy calculations will be necessary to validate and refine the predicted binding modes.

CONCLUSION

From the available 481 nonflavonoid polyphenols, we nominate dicumarol and DWL, which are commercially available, as potential anti-HPV induced cervical cancer compounds. We conclude this through a simulation analysis showing their potential disruptive effect on the E6-p53 binding site. We hypothesize that further effects through their interaction with

ROSQ1 and NQO1 may be beneficial. Although ADMET suggests that they are predicted to be absorbed orally and unlikely to have worse systemic side effects than existing drugs, this still needs to be validated with *in vivo* data. Dicumarol and DWL have been explored, with interesting success, in other cancer types, but not yet in cervical cancer. Thus, we recommend further molecular dynamics and *in vitro* assays of both compounds to validate their potential, especially for dicumarol.

ACKNOWLEDGMENT

This study was supported by the Commission on Higher Education - Philippine-California Advanced Research Institutes (CHED-PCARI) under the grant IHITM 2018-033 "Joint Project for Disease Modeling and Developing Novel Therapeutics: Initial Focus on Acute Myocardial Infarction, Blood Calibration", as well as the Department of Science and Technology of the Philippines under the grant awarded to the "Philippine Program for Diagnostic Biomarkers, Disease Modeling and Nutriceutical Product Development Project 2 (Phil-DIAMOND Project 2)".

CONFLICT OF INTEREST

We report no potential conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Honeylaine O. Arcilla: Conceptualization, experimental assays, data analysis, manuscript draft, manuscript revision
Conrad Allan Chong: Conceptualization, experimental assays, data analysis, manuscript revision
Rod Vincent Borromeo: Conceptualization, experimental assays, manuscript revision
Jessa Louise Cadacio: data analysis, manuscript revision
Ranelle Janine L. Asi: manuscript draft, manuscript revision
Jeffrey Clement C. Bungar: manuscript draft, manuscript revision
Javier Lozano-Gerona: Conceptualization, manuscript draft, manuscript revision, leadership
Ahmad Reza F. Mazahery: Conceptualization, manuscript revision, leadership

REFERENCES

Abbaszadeh, H., Keikhaei, B., & Mottaghi, S. (2019). A review of molecular mechanisms involved in anticancer and antiangiogenic effects of natural polyphenolic compounds. Phytotherapy Research, 33(8), 2002–2014. https://doi.org/10.1002/PTR.6403

Abotaleb, M., Liskova, A., Kubatka, P., & Büsselberg, D. (2020). Therapeutic potential of plant phenolic acids in the treatment of cancer. Biomolecules, 10(2), 221. https://doi.org/10.3390/biom10020221

Adasme, M. F., Linnemann, K. L., Bolz, S. N., Kaiser, F., Salentin, S., Haupt, V. J., & Schroeder, M. (2021). PLIP 2021: expanding the scope of the protein–ligand interaction profiler to DNA and RNA. Nucleic Acids Research, 49(W1), W530–W534. https://doi.org/10.1093/nar/gkab294

Alvarez, M. R. S., Grijaldo, S. J. B., Nacario, R. C., Rabajante, J. F., Heralde III, F. M., Lebrilla, C. B., & Completo, G. C. (2021). In silico screening-based discovery of inhibitors against glycosylation proteins dysregulated in cancer. *Journal of Biomolecular Structure and Dynamics*, 41(5), 1540-1552. https://doi.org/10.1080/07391102.2021.2022534

Amin M. L. (2013). P-glycoprotein Inhibition for Optimal Drug Delivery. *Drug target insights*, *7*, 27–34. https://doi.org/10.4137/DTI.S12519

Asher, G., Lotem, J., Kama, R., Sachs, L., & Shaul, Y. (2002). NQO1 stabilizes p53 through a distinct pathway. Proceedings of the National Academy of Sciences of the United States of America, 99(5), 3099–3104. https://doi.org/10.1073/pnas.052706799

Baghdadi, M. A., Al-Abbasi, F. A., El-Halawany, A. M., Aseeri, A. H., & Al-Abd, A. M. (2018). Anticancer profiling for coumarins and related O-naphthoquinones from Mansonia gagei against solid tumor cells in vitro. *Molecules*, 23(5), 1020. https://doi.org/10.3390/molecules23051020

Bernard, X., Robinson, P., Nominé, Y., Masson, M., Charbonnier, S., Ramirez-Ramos, J. R., Deryckere, F., Travé, G., & Orfanoudakis, G. (2011). Proteasomal Degradation of p53 by Human Papillomavirus E6 Oncoprotein Relies on the Structural Integrity of p53 Core Domain. PLOS ONE, 6(10), e25981. https://doi.org/10.1371/journal.pone.0025981

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000). The Protein Data Bank. Nucleic Acids Research, 28, 235-242. https://doi.org/10.1093/nar/28.1.235

Bosc, N., Atkinson, F., Felix, E., Gaulton, A., Hersey, A., & Leach, A. R. (2019). Large-scale comparison of QSAR and conformal prediction methods and their applications in drug discovery. Journal of Cheminformatics, 11(1), 4. https://doi.org/10.1186/S13321-018-0325-4

Bulusu, G., & Desiraju, G. R. (2020). Strong and weak hydrogen bonds in protein– ligand recognition. Journal of the Indian Institute of Science, 100(1), 31-41. https://doi.org/10.1007/S41745-019-00141-9

Buranrat, B., Prawan, A., Kukongviriyapan, U., Kongpetch, S., & Kukongviriyapan, V. (2010). Dicoumarol enhances gemcitabine-induced cytotoxicity in high NQO1-expressing cholangiocarcinoma cells. World Journal of Gastroenterology, 16(19), 2362-2370. https://doi.org/10.3748/wjg.v16.i19.2362

Burlingham, B. T., & Widlanski, T. S. (2003). An Intuitive Look at the Relationship of Ki and IC50: A More General Use for the Dixon Plot. Journal of Chemical Education, 80(2), 214–218. https://doi.org/10.1021/ed080p214

Celegato, M., Messa, L., Goracci, L., Mercorelli, B., Bertagnin, C., Spyrakis, F., Suarez, I., Cousido-Siah, A., Travé, G., Banks, L., Cruciani, G., Palù, G., & Loregian, A. (2020). A novel small-molecule inhibitor of the human papillomavirus E6- p53 interaction that reactivates p53 function and blocks cancer cells growth. Cancer Letters, 470, 115–125. https://doi.org/10.1016/j.canlet.2019.10.046

Chakraborty, S., & Rahman, T. (2012). The difficulties in cancer treatment. *Ecancermedicalscience*, 6, ed16. https://doi.org/10.3332/ecancer.2012.ed16

Chatterjee, K., AlSharif, D., Mazza, C., Syar, P., Al Sharif, M., & Fata, J. E. (2018). Resveratrol and pterostilbene exhibit anticancer properties involving the downregulation of HPV

oncoprotein E6 in cervical cancer cells. *Nutrients*, 10(2), 243. https://doi.org/10.3390/nu10020243

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S. M., & Savidge, T. C. (2016). Regulation of protein-ligand binding affinity by hydrogen bond pairing. Science Advances, 2(3), e1501240. https://doi.org/10.1126/SCIADV.1501240

Cheng, F., Yu, Y., Zhou, Y., Shen, Z., Xiao, W., Liu, G., Li, W., Lee, P. W., & Tang, Y. (2011). Insights into molecular basis of cytochrome p450 inhibitory promiscuity of compounds. Journal of chemical information and modeling, 51(10), 2482–2495. https://doi.org/10.1021/ci200317s

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *Journal of chemical information and modeling*, *52*(11), 3099–3105. https://doi.org/10.1021/ci300367a

Cherry, J. J., Rietz, A., Malinkevich, A., Liu, Y., Xie, M., Bartolowits, M., Davisson, V. J., Baleja, J. D., & Androphy, E. J. (2013). Structure Based Identification and Characterization of Flavonoids That Disrupt Human Papillomavirus-16 E6 Function. PLOS ONE, 8(12), e84506. https://doi.org/10.1371/journal.pone.0084506

Coimbra, J. T. S., Feghali, R., Ribeiro, R. P., Ramos, M. J., & Fernandes, P. A. (2020). The importance of intramolecular hydrogen bonds on the translocation of the small drug piracetam through a lipid bilayer. RSC Advances, 11(2), 899–908. https://doi.org/10.1039/D0RA09995C

Denny L. (2015). Control of cancer of the cervix in low- and middle-income countries. *Annals of surgical oncology*, *22*(3), 728–733. https://doi.org/10.1245/s10434-014-4344-8

de Sanjose, S., Quint, W. G. V., Alemany, L., Geraets, D. T., Klaustermeier, J. E., Lloveras, B., Tous, S., Felix, A., Bravo, L. E., Shin, H. R., Vallejos, C. S., de Ruiz, P. A., Lima, M. A., Guimera, N., Clavero, O., Alejo, M., Llombart-Bosch, A., ChengYang, C., Tatti, S. A., … Bosch, F. X. (2010). Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *The Lancet Oncology*, 11(11), 1048–1056. https://doi.org/10.1016/S1470- 2045(10)70230-8

Divine, L. M., Kizer, N. T., Hagemann, A. R., Pittman, M. E., Chen, L., Powell, M. A., ... & Thaker, P. H. (2016). Clinicopathologic characteristics and survival of patients with gynecologic malignancies metastatic to the brain. Gynecologic oncology, 142(1), 76-82. https://doi.org/10.1016/j.ygyno.2016.04.030

Drilon, A., Jenkins, C., Iyer, S., Schoenfeld, A., Keddy, C., & Davare, M. A. (2021). ROS1- dependent cancers — biology, diagnostics and therapeutics. Nature Reviews Clinical Oncology, 18(1), 35–55. https://doi.org/10.1038/s41571-020-0408-9

Du, J., Daniels, D. H., Asbury, C., Venkataraman, S., Liu, J., Spitz, D. R., Oberley, L. W., & Cullen, J. J. (2006). Mitochondrial production of reactive oxygen species mediate dicumarol- induced cytotoxicity in cancer cells. Journal of Biological Chemistry, 281(49), 37416–37426. https://doi.org/10.1074/jbc.m605063200

Dulsat, J., López-Nieto, B., Estrada-Tejedor, R., & Borrell, J. I. (2023). Evaluation of Free Online ADMET Tools for Academic or Small Biotech Environments. *Molecules*, *28*(2), 776. https://doi.org/10.3390/molecules28020776

D'Angelo, A., Sobhani, N., Chapman, R., Bagby, S., Bortoletti, C., Traversini, M., Ferrari, K., Voltolini, L., Darlow, J., & Roviello, G. (2020). Focus on ROS1- Positive Non-Small Cell Lung Cancer (NSCLC): Crizotinib, Resistance Mechanisms and the Newer Generation of Targeted Therapies. Cancers, 12(11), 3293. https://doi.org/10.3390/cancers12113293

Evans, W., Filippova, M., Filippov, V., Bashkirova, S., Zhang, G., Reeves, M. E., & Duerksen-Hughes, P. (2016). Overexpression of HPV16 E6* Alters β-Integrin and Mitochondrial Dysfunction Pathways in Cervical Cancer Cells. Cancer genomics & proteomics, 13(4), 259–273.

Gigliarelli, G., Zadra, C., Cossignani, L., Robles Zepeda, R. E., Rascon-Valenzuela, L. A., Velazquez-Contreras, C. A., & Marcotullio, M. C. (2018). Two new lignans from the resin of *Bursera microphylla* A. gray and their cytotoxic activity. *Natural Product Research*, 32(22), 2646-2651. https://doi.org/10.1080/14786419.2017.1375922

Gomes, D., Silvestre, S., Duarte, A. P., Venuti, A., Soares, C. P., Passarinha, L., & Sousa, Â. (2021). In Silico Approaches: A Way to Unveil Novel Therapeutic Drugs for Cervical Cancer Management. *Pharmaceuticals* , 14(8), 741. https://doi.org/10.3390/ph14080741

Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., Liu, G., & Tang, Y. (2018). ADMET-score - a comprehensive scoring function for evaluation of chemical drug-likeness. *MedChemComm*, *10*(1), 148–157. https://doi.org/10.1039/c8md00472b

Gupta, N., Nehra, P., Chauhan, A. S., Mehra, N., Singh, A., Krishnamurthy, M. N., Rajsekhar, K., Kalaiyarasi, J. P., Roy, P. S., Malik, P. S., Mathew, A., Malhotra, P., Kataki, A. C., Dixit, J., Gupta, S., Kumar, L., & Prinja, S. (2022). Cost Effectiveness of Bevacizumab Plus Chemotherapy for the Treatment of Advanced and Metastatic Cervical Cancer in India-A Model-Based Economic Analysis. *JCO global oncology*, *8*, e2100355. https://doi.org/10.1200/GO.21.00355

Hancock, G., Hellner, K., & Dorrell, L. (2018). Therapeutic HPV vaccines. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 47(1), 59–72. https://doi.org/10.1016/j.bpobgyn.2017.09.008

Haupt, L. J., Kazmi, F., Ogilvie, B. W., Buckley, D. B., Smith, B. D., Leatherman, S., Paris, B., Parkinson, O., & Parkinson, A. (2015). The Reliability of Estimating Ki Values for Direct, Reversible Inhibition of Cytochrome P450 Enzymes from Corresponding IC50 Values: A Retrospective Analysis of 343 Experiments. Drug Metabolism and Disposition, 43(11), 1744–1750. https://doi.org/10.1124/dmd.115.066597

Irwin, J. J., & Shoichet, B. K. (2005). ZINC – A Free Database of Commercially Available Compounds for Virtual Screening. Journal of Chemical Information and Modeling, 45(1), 177–182. https://doi.org/10.1021/ci049714

Johnson-Arbor K. &Dubey R. (2023, August 8) Doxorubicin. In StatPearls. StatPearls Publishing. Retrieved May 8, 2025 from https://www.ncbi.nlm.nih.gov/books/NBK459232/

Klempner, S. J., & Ou, S.-H. (2015). Gene Section Short Communication ROS1 (ROS proto-oncogene 1, receptor tyrosine kinase). Atlas of Genetics and Cytogenetics in

Oncology and Haematology, 19(5), 337-339. https://doi.org/10.4267/2042/62121

Kolluru, S., Momoh, R., Lin, L., Mallareddy, J. R., & Krstenansky, J. L. (2019). Identification of potential binding pocket on viral oncoprotein HPV16 E6: A promising anti-cancer target for small molecule drug discovery. BMC Molecular and Cell Biology, 20(1), 30. https://doi.org/10.1186/S12860-019-0214-3

Kumar, A., Rathi, E., & Kini, S. G. (2019). Identification of E6 Inhibitors Employing Energetically Optimized Structure-Based Pharmacophore Modelling, Ligand Docking and Molecular Dynamics Simulations Studies. ChemistrySelect, 4(36), 10701–10708. https://doi.org/10.1002/slct.201902105

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. Journal of Chemical Information and Modeling, 51(10), 2778– 2786. https://doi.org/10.1021/ci200227u

Lee, Y. J., Lin, W. L., Chen, N. F., Chuang, S. K., & Tseng, T. H. (2012). Demethylwedelolactone derivatives inhibit invasive growth *in vitro* and lung metastasis of MDA-MB-231 breast cancer cells in nude mice. European Journal of Medicinal Chemistry, 56, 361–367. https://doi.org/10.1016/j.ejmech.2012.07.041

Li, Q., & Shah, S. (2017). *Structure-Based Virtual Screening.* In Wu, C., Arighi, C., & Ross, K., (Eds.), *Protein Bioinformatics: Methods in Molecular Biology* (Vol. 1558*, pp. 111–124).* Humana Press. doi:10.1007/978-1-4939-6783-4_5

Li, S., Hong, X., Wei, Z., Xie, M., Li, W., Liu, G., Guo, H., Yang, J., Wei, W., & Zhang, S. (2019). Ubiquitination of the HPV oncoprotein E6 is critical for E6/E6AP-mediated p53 degradation. Frontiers in Microbiology, 10, 2483. https://doi.org/10.3389/fmicb.2019.02483

Liu, Z., Wang, S., & Hu, M. (2009). Oral Absorption Basics: Pathways, Physico-chemical and Biological Factors Affecting Absorption. In Qiu, Y., Chen, Y., Zhang, G. G. Z., Liu, L., & Porter, W. R., (Eds.), Developing Solid Oral Dosage Forms, (pp. 263–288). Elsevier. https://doi.org/10.1016/B978-0-444-53242-8.00011-4

Ma, Y., Kong, J., Yan, G., Ren, X., Jin, D., Jin, T., Lin, L., & Lin, Z. (2014). NQO1 overexpression is associated with poor prognosis in squamous cell carcinoma of the uterine cervix. BMC Cancer, 14(1), 414. https://doi.org/10.1186/1471-2407-14-414

Mamgain, S., Sharma, P., Pathak, R. K., & Baunthiyal, M. (2015). Computer aided screening of natural compounds targeting the E6 protein of HPV using molecular docking. Bioinformation, 11(5), 236. https://doi.org/10.6026/97320630011236

Martinez-Zapien, D., Ruiz, F. X., Poirson, J., Mitschler, A., Ramirez, J., Forster, A., Cousido- Siah, A., Masson, M., Pol, S. vande, Podjarny, A., Travé, G., & Zanier, K. 48 (2016). Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53. *Nature*, 529(7587), 541–545. https://doi.org/10.1038/nature16481

Matsson, P., & Kihlberg, J. (2017). How Big Is Too Big for Cell Permeability? Journal of medicinal chemistry, 60(5), 1662–1664. https://doi.org/10.1021/acs.jmedchem.7b00237

Matsui, Y., Watanabe, J., Ding, S., Nishizawa, K., Kajita, Y., Ichioka, K., Saito, R., Kobayashi, T., Ogawa, O., & Nishiyama, H. (2010). Dicoumarol enhances doxorubicin-induced cytotoxicity in p53 wild-type urothelial cancer cells through p38 activation. BJU International, 105(4), 558–564. https://doi.org/10.1111/j.1464-410x.2009.08732.x

Mendez, D., Gaulton, A., Bento, A. P., Chambers, J., de Veij, M., Félix, E., Magariños, M. P., Mosquera, J. F., Mutowo, P., Nowotka, M., Gordillo-Marañón, M., Hunter, F., Junco, L., Mugumbate, G., Rodriguez-Lopez, M., Atkinson, F., Bosc, N., Radoux, C. J., Segura- Cabrera, A., … Leach, A. R. (2019). ChEMBL: towards direct deposition of bioassay data. Nucleic Acids Research, 47(D1), D930–D940. https://doi.org/10.1093/nar/gky1075

Moga, M. A., Dimienescu, O. G., Arvatescu, C. A., Mironescu, A., Dracea, L., & Ples, L. (2016). The Role of Natural Polyphenols in the Prevention and Treatment of Cervical Cancer—An Overview. *Molecules*, 21(8), 1055. https://doi.org/10.3390/molecules21081055

Molinspiration Cheminformatics. (n.d.). Molinspiration Cheminformatics free web services. Retrieved from https://www.molinspiration.com

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Nabati, F., Moradi, M., & Mohabatkar, H. (2020). In silico analyzing the molecular interactions of plant-derived inhibitors against E6AP, p53, and c-Myc binding sites of HPV type 16 E6 oncoprotein. Molecular Biology Research Communications, 9(2), 71-82. https://doi.org/10.22099/mbrc.2020.36522.1483

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of cheminformatics, 3(1), 33. https://doi.org/10.1186/1758-2946-3-33

Pal, A., & Kundu, R. (2020). Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. Frontiers in Microbiology, 10, 3116. https://doi.org/10.3389/fmicb.2019.03116

Park, W. H. (2017). Gallic acid induces HeLa cell death via increasing GSH depletion rather than ROS levels. *Oncology Reports*, 37(2), 1277–1283. https://doi.org/10.3892/OR.2016.5335

Prakash, S., Elavarasan, N., Subashini, K., Kanaga, S., Dhandapani, R., Sivanandam, M., Kumaradhas, P., Thirunavukkarasu, C., & Sujatha, V. (2020). Isolation of hesperetin - A flavonoid from Cordia sebestena flower extract through antioxidant assay guided method and its antibacterial, anticancer effect on cervical cancer via *in vitro* and in silico molecular docking studies. Journal of Molecular Structure, 1207, 127751. https://doi.org/10.1016/j.molstruc.2020.127751

Roskoski, R. (2017). ROS1 protein-tyrosine kinase inhibitors in the treatment of ROS1 fusion protein-driven non-small cell lung cancers. Pharmacological Research, 121, 202–212. https://doi.org/10.1016/J.PHRS.2017.04.022

Sterling, T., & Irwin, J. J. (2015). ZINC 15 - Ligand Discovery for Everyone. Journal of Chemical Information and Modeling, 55(11), 2324–2337. https://doi.org/10.1021/ACS.JCIM.5B00559

Sun, C., Zhao, W., Wang, X., Sun, Y., & Chen, X. (2020). A pharmacological review of dicoumarol: An old natural anticoagulant agent. Pharmacological Research, 160, 105193. https://doi.org/10.1016/J.PHRS.2020.105193

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Syed, S. D., Deepak, M., Yogisha, S., Chandrashekar, A. P., Muddarachappa, K. A., D'Souza, P., Agarwal, A., & Venkataraman, B. v. (2003). Trypsin inhibitory effect of wedelolactone and demethylwedelolactone. Phytotherapy Research, 17(4), 420– 421. https://doi.org/10.1002/PTR.1153

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461. https://doi.org/10.1002/JCC.21334

Van Breemen, R. B., & Li, Y. (2005). Caco-2 cell permeability assays to measure drug absorption. Expert opinion on drug metabolism & toxicology, 1(2), 175–185. https://doi.org/10.1517/17425255.1.2.175

Wang, S., Li, Y., Wang, J., Chen, L., Zhang, L., Yu, H., & Hou, T. (2012). ADMET evaluation in drug discovery. 12. Development of binary classification models for prediction of hERG potassium channel blockage. *Molecular pharmaceutics*, *9*(4), 996-1010. https://doi.org/10.1021/mp300023x

Watanabe, J., Nishiyama, H., Matsui, Y., Ito, M., Kawanishi, H., Kamoto, T., & Ogawa, O. (2006). Dicoumarol potentiates cisplatin-induced apoptosis mediated by c-Jun N-terminal kinase in p53 wild-type urogenital cancer cell lines. Oncogene, 25(17), 2500–2508. https://doi.org/10.1038/sj.onc.1209162

Wishart DS, Tian S, Allen D, Oler E, Peters H, Lui VW, Gautam V, Djoumbou-Feunang Y, Greiner R, Metz TO. BioTransformer 3.0-a web server for accurately predicting metabolic transformation products. Nucleic Acids Res. 2022 Jul 5;50(W1):W115-W123. doi: 10.1093/nar/gkac313. PMID: 35536252; PMCID: PMC9252798.

Wong, S. E., & Lightstone, F. C. (2011). Accounting for water molecules in drug design. Expert opinion on drug discovery, 6(1), 65-74. https://doi.org/10.1517/17460441.2011.534452

Yang, N. J., & Hinner, M. J. (2015). Getting Across the Cell Membrane: An Overview for Small Molecules, Peptides, and Proteins. In Gautier, A. & Hinner, M., (Eds.), Site-Specific Protein Labeling: Methods in Molecular Biology, (Vol. 1266, pp. 29-53). https://doi.org/10.1007/978-1-4939-2272-7_3

Yang, H., Lou, C., Sun, L., Li, J., Cai, Y., Wang, Z., Li, W., Liu, G., & Tang, Y. (2019). admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics*, *35*(6), 1067–1069. https://doi.org/10.1093/bioinformatics/bty707

Zanier, K., Stutz, C., Kintscher, S., Reinz, E., Sehr, P., Bulkescher, J., Hoppe-Seyler, K., Travé, G., & Hoppe-Seyler, F. (2014). The E6AP Binding Pocket of the HPV16 E6 Oncoprotein Provides a Docking Site for a Small Inhibitory Peptide Unrelated to E6AP, Indicating Druggability of E6. PLOS ONE, 9(11), e112514. https://doi.org/10.1371/journal.pone.0112514

Zhou, Y., Zheng, J., Li, Y., Xu, D. P., Li, S., Chen, Y. M., & Li, H. bin. (2016). Natural Polyphenols for Prevention and Treatment of Cancer. *Nutrients*, 8(8), 515. https://doi.org/10.3390/NU8080515

SUPPLEMENTARY INFORMATION

Supplementary Table 1: Top 14 compounds with the strongest binding affinity to HPV16 E6, predicted pharmacodynamic parameters, and protein-ligand interaction results. A more negative binding affinity value favors greater attraction and hence stronger affinity.

	LIGAND	ΔG (kcal/ mol)[a]	pKi (μM)[b]	pIC50 (μM)[c]	INTERACTING RESIDUES	No. of H-bonds
1	Picropolygamain (Analog 1)	-9.1	0.21	0.43	Tyr32, Leu50, Cys51, Phe45, Val53, Val62, Val31, Ser71, Ser74, Gln107, Arg131, Leu67, Arg102	**2**
2	Gerberinol	-8.9	0.30	0.60	Tyr32, Leu50, Cys51, Phe45,Val62, Ser71, Ser74, Gln107,Arg131, Tyr70, Leu67	**1**
3	**Dicumarol**	**-8.8**	**0.35**	**0.71**	**Leu50, Cys51, Val62, Leu76,Arg131, Gln107**	**2**
4	Calanolide A(Analog 1)	-8.7	0.42	0.84	Leu50, Cys51, Tyr32, Val53,Val62, Leu67, Tyr70, Ser71,Gln107, Arg102	**2**
5	7-Hydroxyenterolactone	-8.6	0.50	0.99	Tyr32, Tyr70, Ser71, Val31, Val62, Ala61, Cys51, Phe45,Val53, Leu67, Ser74, Leu50, Gln107, Arg131, Arg102	**4**
6	Calanolide A(Analog 2)	-8.6	0.50	0.99	Leu50, Cys51, Tyr70, Val53, Tyr32, Cys66, Val62, Leu67, Ser71, Ser74, Gln107, Arg102	**4**
7	Psoralidin	-8.6	0.50	0.99	Ala61, Val62, Val53, Val31,Tyr32, Ser71, Tyr70, Leu67,Arg131, Gln107, Arg102	**2**
8	Felamidin	-8.5	0.59	1.18	Leu50, Cys51, Phe45, Tyr32, Val62, Val131, Leu67, Val53,Ser71, Arg131, Arg102	**0**
9	Picropolygamain (Analog 2)	-8.5	0.59	1.18	Leu50, Cys51, Tyr70, Val53,Val62, Phe45, Leu67, Tyr32,Ser71, Ser74	**2**
10	Llagate	-8.4	0.70	1.39	Leu50, Val53, Val31, Tyr32,Leu67, Tyr70, Ser74, Ser71,Gln107	**5**
11	5-Methoxyhinokinin	-8.4	0.70	1.39	Ser71, Ser74, Leu67, Tyr32, Tyr70, Val53, Val31, Leu60, Val62, Arg102, Gln107, Arg131	**1**
12	Ammoresinol	-8.3	0.82	1.65	Leu50, Cys51, Val53, Ala61,Val31, Val62, Ser71, Leu67, Tyr70, Tyr32, Ser74, Gln107, Arg131	**0**

13	**Demethylwedelolactone (DWL)**	**-8.3**	**0.82**	**1.65**	**Leu50, Cys51, Leu67, Tyr70,Ser71, Tur32, Ser74, Gln107,Arg131**	**3**
14	Fraxine (Analog1)	-8.3	0.82	1.65	Ser71, Ser74, Tyr32, Leu67,Leu50, Gln107, Arg131	**4**
Control	**Daphnoretin**	**-8.3**	**0.82**	**1.65**	**Val69, Leu74, Leu57, Tyr77,Ser81, Ile135**	**5**

Supplementary Table 2: ADMET Profile of the Tested Compounds: Dicumarol, DWL, Daphnoretin, and Doxorubicin.

	Dicumarol	**DWL**	**Daphnoretin**	**Doxorubicin**
Ames mutagenesis	-	-	-	+
Acute Oral Toxicity (c)	II	II	III	III
Androgen receptor binding	+	+	+	+
Aromatase binding	+	+	+	+
Avian toxicity	-	-	-	-
Blood Brain Barrier	-	-	-	-
BRCP inhibitior	-	-	-	-
Biodegradation	-	-	-	-
BSEP inhibitior	-	-	-	+
Caco-2	-	-	-	-
Carcinogenicity (binary)	-	-	-	-
Carcinogenicity (trinary)	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	+	+	+	+
Fish aquatic toxicity	+	+	+	+
Glucocorticoid receptor binding	+	+	+	+
Honey bee toxicity	-	-	-	-
Hepatotoxicity	+	-	-	-
Human Ether-a-go-go-Related Gene inhibition	-	-	-	-
Human Intestinal Absorption	+	+	+	-
Human oral bioavailability	+	-	+	-
MATE1 inhibitior	-	-	-	-
Mitochondrial toxicity	+	+	+	+
Micronuclear	+	+	+	+
Nephrotoxicity	-	-	+	+
Acute Oral Toxicity	2.247183323	1.574755073	1.216175079	3.228397846

OATP1B1 inhibitior	-	+	+	+
OATP1B3 inhibitior	+	+	+	+
OATP2B1 inhibitior	-	-	-	-
OCT1 inhibitior	-	-	-	-
OCT2 inhibitior	-	-	-	-
P-glycoprotein inhibitior	-	-	+	-
P-glycoprotein substrate	-	-	-	+
PPAR gamma	+	+	+	+
Plasma protein binding	0.902910948	1.006513119	0.998371542	0.621866405
Reproductive toxicity	+	+	+	+
Respiratory toxicity	+	+	-	+
skin sensitisation	-	-	-	-
Subcellular localzation	Mitochondria	Mitochondria	Mitochondria	Nucleus
Tetrahymena pyriformis	1.354358435	2.079515219	2.097493887	0.616532207
Thyroid receptor binding	-	+	-	-
UGT catelyzed	+	+	+	+
Water solubility	-3.172360133	-2.945803968	-3.530027858	-2.719094044