

RESEARCH ARTICLE

In silico assessment of the binding of reported bioactive compounds from *Phaseolus vulgaris* L. (Fabaceae) towards the wild-type and mutant estrogen receptor- α

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¹Department of Pharmaceutical Chemistry, College of Pharmacy, University of the Philippines Manila, Manila, Philippines²Department of Physical Sciences and Mathematics, College of Arts and Sciences, University of the Philippines Manila, Manila, Philippines**ABSTRACT**

Background: Breast cancer is one of the leading causes of deaths in women worldwide, affecting nearly 7.8 million people. In 2020 in the Philippines, there were around 150,000 Filipinos who were newly diagnosed with the disease. The complex pathogenesis of breast cancer in addition to the emergence of resistance to therapy makes the treatment very challenging. Compounds that can antagonize the effects of estradiol towards ER- α , especially the mutant Y537S type are sought for.

Objectives: The focus of this study was the *in-silico* assessment of the reported secondary metabolites from *Phaseolus vulgaris* L. (fam. Fabaceae) towards the wild-type and mutant ER- α . Bioisosteric replacement was conducted to generate analogs that can possibly have a comparable binding affinity as estradiol towards estrogen receptors alpha.

Results: Majority of the secondary metabolites present in *Phaseolus vulgaris* L. belong to the group of phytoestrogens, phytosterols, and plant hormones. These groups of compounds exhibited favorable binding energies toward the wild-type and mutant (Y537S) estrogen receptors alpha. Moreover, they bind to the same ligand binding pocket as estradiol, involving similar interactions and amino acid residues.

Conclusion: Compounds from *Phaseolus vulgaris* L. can potentially target ER- α . Four gibberellin A19 analogs were generated that exhibited favorable binding towards the wild- and mutant- ER- α and may be further optimized to obtain a promising compound against breast cancer.

Keywords: breast cancer, ER- α , *Phaseolus vulgaris* L., *in silico* screening, molecular docking, Bagoio beans

Introduction

Breast cancer is a collection of malignancies which affect the mammary gland and may metastasize to other parts of the body. It is a multifactorial disease involving estrogen, estrogen receptors, and inflammatory response, among others. It affects nearly 2.1 million women yearly and accounts for about 600,000 deaths in 2018 [1,2]. From 2015 to 2020, 7.8 million women were diagnosed with the disease, making it the most prevalent type of cancer worldwide [3]. In the Philippines, breast cancer is the most common type of cancer, leading to more than 200,000 new cases in both sexes and an estimated 7000 deaths [4]. It has affected around 350,000 Filipinos (male and female) in the last five years. In the year 2020, there are around 150,000 Filipinos who were newly diagnosed with the disease [2].

Several risk factors such as being female, age of menopause, genetic predisposition involving BRCA1 and BRCA2, early menarche, and high levels of endogenous sex hormones are attributed to the development of breast cancer [5-7].

About 70% of breast cancer patients test positive for ER- α , the receptor which is responsible for enhanced cellular proliferation the activation of which contributes to the pathogenesis of the disease [8]. ER- α is a transcription factor encoded by the ESR1 gene located at chromosome number 6. Mutations in the ESR1 gene are observed in metastatic breast cancers that are previously treated with aromatase inhibitors and are associated with a recalcitrant disease and worse prognosis. The most common site of mutation is in

the ligand binding domain at Y537 (tyrosine-537 residue) and D538 (aspartic acid-538 residue). These mutations result in a constitutively active receptor, independent of estrogen [10]. The Y537S (tyrosine 537 residue replaced with serine) and the D538G (aspartic acid replaced with glycine) mutations are prevalent and results in a reduced affinity of both tamoxifen and estrogen on the mutant receptors [8,10,15,16]. Other mutations may lead to estrogen hypersensitivity (K303R—lysine 303 replaced with arginine; E380Q—glutamate 380 replaced with glutamine) or a retained estrogen-dependent activity (S432L—serine 432 replaced with lysine; V534E—valine 534 replaced with glutamate) [10]. Among the mutations, the Y537S isoform is the most aggressive type with a 20-month survival rate [25].

While the pathophysiology of breast cancer is complex, Joshi and Press (2018) summarized the hallmarks of cancer which include sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, induction of angiogenesis, activation of invasion and metastasis, evasion of immune destruction, reprogramming of bioenergetics, tumor-promoting inflammation, and genome instability and mutation which enables replicative immortality [13]. These hallmarks have been targets of breast cancer research and the identified biomarkers such as the estrogen receptors, progesterone receptors, and human epidermal growth factor (HER2) are keys in the current therapy [9].

Several treatment options for breast cancer are available depending on the presence or absence of markers such as ER- α and HER-2. These include the anti-estrogenic drug tamoxifen, anti-aromatase drugs such as exemestane and letrozole, and other chemotherapy drugs like taxanes and doxorubicin, and the anti-HER2 drugs trastuzumab and pertuzumab [6]. However, development of resistance is possible [14] and toxicities such as asthenia, myelosuppression, and disruption of sensory signals are observed with the current treatments [6]. Mutations in the ESR1 gene which codes for the ER- α acquired from previous treatments with aromatase inhibitors is one of the mechanisms by which resistance and a recalcitrant disease develops. Hence, one of the strategies against breast cancer is to target the mutant ER- α . Moreover, adjunct treatments or key treatments with few to no side effects are continuously sought for [8,10,15,16].

Several studies have shown the potential of *Phaseolus vulgaris* L. (PVL) against cancer based on the dose-activity relationships [17-21]. *Phaseolus vulgaris* L. belongs to the family of legumes and is very common in the Philippines. The bean is a good source of protein, nutrients, and phytochemicals. Abu-

Reidah, *et al.* (2012) reported that most of the phenolics found in the bean are flavonoids and flavonoid derivatives [22]. These flavonoids belong to the phytoestrogens group of compounds which are nonsteroidal secondary metabolites having structural similarity and hence bioactivity as that of endogenous estrogens. Because of their ability to interact with the estrogen receptors, they can elicit estrogenic and antiestrogenic effects. These include modulation of bone homeostasis, cardiovascular effects, and reproductive effects [23]. Additionally, the beans contain phaseolin and lectin which are proteins that exhibit biological activities such as agglutination, mitosis, and cell growth inhibition [20].

The numerous bioactivities of the compounds from *Phaseolus vulgaris* L. make it a promising natural product against breast cancer. This study focused on *in silico* screening of the secondary metabolites from *Phaseolus vulgaris* L. against the wild-type and mutant ER- α . Bioisosteric replacement in the structure of hit compounds was utilized as a strategy to determine analogs that may possibly have a better binding energy than the parent compounds. The present study may provide insights on how the bioactive compounds of *Phaseolus vulgaris* L. (Fabaceae) bind to ER- α , especially the mutant receptors.

Methodology

Literature Search of Compounds from Phaseolus vulgaris L. (PVL) and Initial Screening

Literature search was conducted to identify the compounds that are present in PVL. Research articles which included methods of identification of PVL compounds were used. Only the secondary metabolites were included. For the glycosides, only the aglycone portion was considered for screening since glycosides are too polar to be absorbed via passive diffusion, hence, only the aglycones are likely to be absorbed. Moreover, in the screening process, they either fail the topological polar surface area (a parameter of oral bioavailability) or the drug-likeness filters. ADMETlab 2.0 (open source at <https://admetmesh.scbdd.com/>) was used for the primary screening of compounds [24]. In this study, the SMILES strings were obtained from the PubChem database. Both the canonical SMILES and the isomeric SMILES were used in the web server. The compounds considered for further evaluation were nonmutagenic/noncarcinogenic, have good oral bioavailability or high intestinal absorption, and have passed any of the drug-likeness rules (Lipinski rule, quantitative estimate of drug-likeness).

Protein and Ligand Structure

Thirteen ER- α receptor crystal structures were used in this study. These include one wild-type (PDB ID: 1A52) and twelve mutagenic Y537S receptor models (PDB ID: 3UUD, 4ZNS, 4ZNV, 5DXE, 5DXG, 5KCD, 5KCT, 5KD9, 5TLT, 5TM8, 5TN4, and 5TN5). The crystal structures of the receptors with their corresponding co-crystallized ligands are available at the protein data bank website (<https://www.rcsb.org/>). The Y537S mutation was chosen because it is one of the most common ER- α mutations and is the most aggressive [25]. All the mutant crystal structures have a resolution of 1.5 Å -2 Å whereas the wild-type has a 2.8 Å resolution. These crystal structures were also utilized by Shylaja *et al.* [26].

The 3-D structures of the ligands were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) as SDF file format [27]. For ligands with isomeric SMILES, these were copied and pasted into the Swiss ADME web server to generate the 2-D structures. The 2-D structures generated were then processed using ChemSketch 2021.1.1 (<https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/>) to generate the 3-D structures [29]. These were saved as MOL files. All ligands were converted to PDB files before ligand processing and molecular docking.

Preparation of Protein

All protein crystal structures were downloaded from the protein data bank (www.rcsb.org) as PDB file. The proteins were processed using AutoDockTools v1.5.6 using the standard protocol [30]. Briefly, the file was opened using Autodock tools (ADT) and then water molecules were deleted, nonpolar hydrogens were merged, polar hydrogens were added, co-crystallized ligands, and other molecules not part of the receptor were deleted. Missing residues were repaired and Kollmann charges were then added.

Preparation of PVL Ligands

The ligands files in the SDF and MOL file formats were converted into PDB file format using Open Babel v3.1.1 [31]. The PDB files were then processed using ADT to add charges, merge nonpolar hydrogens, and add polar hydrogens. The final structures of the ligands were checked to ensure that the correct structures were generated because it was observed during the initial preparation steps that the ADT has the tendency to detect closely spaced atoms as bonded atoms. The outputs were in PDBQT file format.

Gridbox Optimization

To obtain the coordinates of the gridbox (search space) that were used in the molecular docking simulation, the proteins (PDB file format) were opened using Discovery Studio Visualizer v21.1.0.20298. In the graphic-user interface, the Receptor-Ligand Interactions tab was clicked, and, in this panel, the receptor was defined, and the site was defined based on PDB records. The active site will appear in the right-hand panel as a sphere. The sphere was clicked, and the attributes were determined. Here, the coordinates of the active site will appear as XYZ coordinates. These coordinates are used in the ADT and the search space was adjusted such that the volume did not exceed 27,000 Å³. Moreover, the ligand binding site was contained within the search space. The final volume used in the search space was 22 x 22 x 22 Å³.

The ligands co-crystallized with the receptors were redocked in the receptor using Autodock Tools v1.5.6 and Autodock Vina v1.1.2. The number of modes was set to 10, the energy range was set to 3, and exhaustiveness was set to 8. It was ensured that the RMSD values with respect to the original pose of the crystallized ligand were less than 2 Å [32]. The grid box validation parameters and results are shown in Supplementary Table 1 and Supplementary Figure 1.

Molecular Docking

All molecular docking simulations were performed using Autodock Vina [32]. The standard protocol of for running Autodock Vina provided by Forli *et al.* (2016) was utilized [30]. The binding affinity (kcal/mol) of each ligand and receptor pair was calculated. The binding affinities of the native ligand estradiol (E2), and the selective estrogen receptor modulator, tamoxifen, were used as the reference values. PVL ligands with binding affinity values that are +2.5 kcal/mol with respect to these reference values were considered in the analyses. This threshold is a conservative estimate of the standard error (2.8 kcal/mol) of the results obtained from Autodock Vina [32]. Also, the binding affinities of the PVL ligands with respect to the 13 receptor models were considered. That is, they have negative binding affinity values across the receptor models and were within the +2.5 kcal/mol binding energy with respect to the reference compounds. Ligands were docked five times to ensure consistency in the binding affinity values. The resulting PDBQT files were visualized using Discovery Studio Visualizer v21.1.0.20298 [33] and the ligand interactions between the different receptor models and the PVL ligands, tamoxifen, and estrogen were noted. To automate molecular docking,

python script, PERL script, and windows command prompt were utilized.

Bioisosteric Replacements to Generate Analogs of PVL Ligands

Bioisosteres of PVL ligands which were included in the final list were generated using MolOpt, an open-source web server (<https://xundrug.cn/molopt>) [33]. The input are SMILES strings and the output are CSV files containing the structures of the bioisosteres as well as the ADMET profile. The web server treats the molecules in two-dimensional configurations; hence, stereoisomers are not recognized. All the bioisosteres generated by the web server were screened by their ADMET profiles. The chosen bioisosteres subjected for molecular docking simulations were nontoxic (liver and kidney cell lines, heart cells, rat, and mouse models) and non-carcinogenic/non-mutagenic, were not substrates/inhibitors/activators of CYP family of enzymes (CYP450 1A2, CYP 450 2C19, CYP450 2C9, CYP450 3A4, CYP 450 2D6), non-inhibitors of p-glycoprotein, and have high intestinal absorption. After the screening, the filtered bioisosteres were then screened again using Swiss ADME to ensure that they have high predicted oral bioavailability. The 2-D structures of the final list of bioisosteres were generated using the Swiss ADME web server [28] and then processed using ChemSketch 2021.1.1 [29]. The 3-D structures were then optimized and the stereoisomers of each bioisostere were generated. The structures of the stereoisomers were 3-D optimized and then subjected to molecular docking simulation. The MOL files output were converted to PDB files using Open Babel.

The final list of bioisosteres which showed comparable binding energies to estrogen and tamoxifen towards the different receptor models were reprocessed using MarvinSketch v21.20.0 [35]. Using this application, 3-D structure optimization was performed to on each structure to find low-energy conformers. Moreover, protonation states of the structures were calculated before doing molecular docking simulations.

Data Analysis

The compounds were grouped according to their compound class which includes flavonoids and phytosterols. The binding energies of the compounds towards each receptor were tabulated and compared. The PDBQT files resulting from the Autodock Vina molecular docking simulation were visualized using Discovery Studio Visualizer v21.1.0.20298 and PyMol v2.5.2 [36]. The ligand-receptor interactions were noted and were related to their binding energies.

Results

The determination of compounds that are found in the edible parts of *Phaseolus vulgaris* L. (PVL) was conducted through literature search. The initial list contains glycosides that have the same aglycone portion but different sugar moieties. For example, quercetin 3-O-glucoside and quercetin 3-O-rutinoside. In these cases, only one of the compounds was chosen to be part of the list and only the aglycone portion was subjected to screening and molecular docking. Supplementary Table 2 summarizes the secondary metabolites found in PVL. It also includes the plant parts where they were identified, the country of origin, the variety, and the references from where they were lifted. The compound class of secondary metabolites found include flavonoids (flavanone, flavanol, flavone, flavonol, isoflavone, flavononol, anthocyanidin), lignan (isolariciresinol), stilbene (resveratrol), phytosterols (e.g., stigmasterol, beta-sitosterol), plant hormones (e.g., gibberellins), cinnamic and benzoic acid derivatives (e.g., quinic acid, ferulic acid, hydroxybenzoic acid), and soyasaponins [37-45].

The compounds were screened using ADMETlab 2.0 open web server and the results were summarized in Supplementary Table 3. HIA (human intestinal absorption), bioavailability (E), carcinogenicity/ mutagenicity/ genotoxicity, QED (quantitative estimate of drug-likeness), and Lipinski's rule of five were used as filters. Compounds must have high intestinal absorption (0-0.3) or must have an oral bioavailability of at least 20-30% (0-0.3), must be noncarcinogenic, nonmutagenic, and nongenotoxic, and must be accepted in any of the drug-likeness rules or have QED value of greater than 0 [24]. Moreover, Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>) was utilized to determine if ER- α is included as a potential target of these compounds.

The secondary metabolites from *Phaseolus vulgaris* L. were then subjected to molecular docking using Autodock Vina v.1.1.2. The final list of compounds which have comparable binding affinities (in kcal per mole) with tamoxifen and estrogen towards the ER- α models (PDB ID: 1A52, 3UUD, 4ZNS, 4ZNV, 5DXE, 5DXG, 5KCD, 5KCT, 5KD9, 5TLT, 5TM8, 5TN4, and 5TN5) were summarized in. The results showed that estrogen exhibits more negative binding affinity across the different receptor models compared to tamoxifen and the PVL compounds. This indicates that estrogen has a better binding affinity than either tamoxifen or PVL compounds. When compared to tamoxifen, most of the PVL compounds exhibit a more negative binding affinity values across the mutant models. On the other hand, tamoxifen outperforms the PVL compounds in terms of

Table 1. Binding Energy Results of Estradiol, Tamoxifen, PVL compounds, and Gibberellin A19 Analogs when Docked to Estrogen Receptors- α .

Compound Class	Compound	1A52	3UUD	4ZNS	4ZNV	5DXE	5DXG	5KCD	5KCT	5KD9	5TLT	5TM8	5TN4	5TN5
Endogenous Ligand	Estradiol	-10.7	-10.9	-9.5	-9.6	-11.2	-11	-9.9	-10.2	-10.1	-10.2	-9.6	-9.9	-11.1
SERM	Tamoxifen	-9.3	-5.1	-9.1	-8.6	-4.6	-4.2	-8.8	-9.2	-8.8	-9.3	-9.8	-4.8	-5.0
Flavanone	naringenin	-8.6	-8.0	-8.7	-9.0	-8.0	-7.6	-8.7	-8.8	-8.6	-8.9	-8.6	-8.6	-8.1
	pinocembrin	-8.4	-7.9	-8.1	-8.3	-8.2	-8.0	-8.0	-8.4	-8.4	-7.9	-7.8	-7.5	-8.2
Flavone	apigenin	-8.7	-7.9	-8.8	-9.1	-7.9	-7.5	-8.7	-8.6	-8.7	-8.8	-8.6	-8.5	-8.0
	luteolin	-8.5	-8.0	-8.7	-8.9	-7.8	-7.6	-8.9	-8.8	-8.3	-8.9	-8.3	-8.8	-8.1
Isoflavone	daidzein	-9.1	-8.4	-8.8	-8.9	-8.5	-8.2	-8.9	-8.4	-7.7	-8.5	-8.6	-8.8	-8.5
Flavononol	aromadendrin	-7.9	-7.7	-8.3	-8.6	-7.9	-7.7	-8.3	-8.4	-8.3	-8.6	-8.2	-8.1	-7.9
Anthocyanidins	cyanidin	-9.0	-8.9	-8.7	-8.6	-8.9	-8.8	-8.7	-8.4	-9	-8.8	-8.6	-8.7	-8.7
Phytosterols	beta-sitosterol	-6.8	-3.8	-7.0	-8.0	-2.9	-2.1	-8.0	-8.4	-3.5	-9.3	-7.3	-2.0	-2.7
	campesterol	-7.1	-5.0	-8.1	-8.7	-4.2	-3.4	-7.8	-9.3	-3.3	-10.1	-8.3	-2.3	-3.9
	delta-7 avenasterol	-7.4	-6.0	-7.4	-8.6	-5.6	-4.3	-9.0	-8.8	-2.5	-9.5	-7.8	-3.1	-5.3
	fucosterol	-6.3	-3.7	-7.8	-8.3	-3.0	-1.6	-7.9	-8.5	-4.1	-9.6	-8.2	-1.5	-2.6
	stigmasterol	-6.5	-2.8	-6.4	-8.2	-2.0	-1.1	-8.3	-8.4	-1.6	-8.6	-7.6	-1.6	-1.6
Gibberellin A19 Analogs	Analog 1	-8.7	-9.1	-8.4	-7.3	-8.8	-8.7	-8.9	-8.2	-8.5	-8.8	-9.1	-8.6	-8.4
	Analog 2	-7.9	-8.0	-7.3	-7.7	-8.1	-8.1	-8.2	-8.6	-7.3	-8.0	-7.0	-7.4	-7.1
	Analog 3	-8.6	-8.9	-8.3	-7.2	-8.5	-8.4	-8.6	-8.1	-8.5	-8.6	-8.9	-8.4	-8.3
	Analog 4	-8.6	-8.5	-7.6	-7.2	-8.2	-7.8	-9.7	-7.5	-7.4	-9.0	-9.1	-7.5	-7.6

binding affinity on the wild-type ER- α . Tamoxifen's binding affinity, however, tends to become less favorable towards the mutant type of ER- α as seen especially in 3UUD (-5.1 kcal/mol), 5DXE (-4.6 kcal/mol), 5DXG (-4.2 kcal/mol), 5TN4 (-4.8 kcal/mol), and 5TN5 (-5.0 kcal/mol). In contrast, most of the PVL compounds have a consistent binding affinity value towards all the receptor models, both the wild-type and the mutant receptors. Notable deviations were observed with the phytosterols. For example, stigmasterol has a binding affinity of -9.1 kcal/mol in the mutant ER- α 5TLT but has -1.6 kcal/mol binding affinity when docked in the 5TN4 model. The same is true with fucosterol, (-9.6 kcal/mol in 5TLT and -1.5 kcal/mol in 5TN4), beta-sitosterol (-9.3 kcal/mol in 5TLT and -2.0 kcal/mol in 5TN4), campesterol (-10.1 in 5TLT and -2.3 kcal/mol in 5TN4), and delta 7-avenasterol (-9.5 kcal/mol in 5TLT and -3.1 kcal/mol in 5TN4). While less favorable binding energies were observed with phytosterols when docked in some of the models, the observed highly negative and comparable binding energies to tamoxifen and estrogen towards the other models, especially the mutant ER- α , cannot be discounted.

Based on the calculated binding energies, none of the PVL compounds nor tamoxifen outperformed estradiol in terms of binding affinity towards the ER- α models. Hence, bioisosteric replacement was used as a strategy to generate analogs of the PVL compounds. MolOpt open web server was utilized to generate the analogs. More than 296,000 bioisosteres were created and from this, 1,572 compounds were subjected to molecular docking. The stereoisomers of these compounds were also generated and were subjected to molecular docking which led to 5,135 compounds. Based on the SWISS ADME Results, some PVL compounds have bioisosteres which did not pass the oral bioavailability criteria and were excluded. Only analogs with favorable binding energies (-7.5 kcal/mol) across the different receptors used in this study were considered. Shown in Table 1 are the four gibberellin A19 analogs as well as their binding energies toward the wild-type and mutant receptors.

To determine the amino acid residues that interact with the ligands, the resulting PDBQT files were viewed using Discovery Studio Visualizer v21.1.0.20298. The results are

Table 2. Protein-ligand interactions between Estradiol, Tamoxifen, *Phaseolus vulgaris* L. compounds, Gibberellin A19 analogs, and the Wild-type ER- α (PDB ID: 1A52).

Compound Class	Compound	Hydrogen Bonding	Hydrophobic Interactions	π - π Stacking Interactions	Miscellaneous interactions	Unfavorable Interactions
Endogenous Ligand	Estradiol	HIS 524	ILE 424, MET 388, LEU 391, LEU 387, ALA 350	PHE 404	-	GLU 353 (unfavorable acceptor-acceptor)
SERM	Tamoxifen	THR 347	ILE 424, MET 388, ALA 350, LEU 525, LEU 391, LEU 387	PHE 404	MET 421(π -S)	-
Flavanone	naringenin	PHE 404, ARG 394	LEU 391, ALA 350, LEU 349, LEU 387, ILE 424	PHE 404	-	-
	pinocembrin	-	LEU 346, LEU 387, ALA 350, LEU 391, LEU 349	PHE 404	GLU 353 (π -anion), MET 421 (π -sulfur)	-
Flavone	apigenin	PHE 404, ARG 394	LEU 349, LEU 391, LEU 387, ALA 350, LEU 384	PHE 404	-	-
	luteolin	PHE 404, GLY 521, ARG 394	LEU 349, LEU 391, ALA 350, LEU 387, LEU 384	PHE 404	-	GLU 353 (acceptor-acceptor)
Isoflavone	daidzein	ARG 394, PHE 404, GLY 521, HIS 524	LEU 346, LEU 391, ALA 350, LEU 387, LEU 525	PHE 404	MET 421 (π -S)	GLU 353 (acceptor-acceptor)
Flavononol	aromadendrin	GLU 353, ARG 394, LEU 346, THR 347	MET 421, LEU 387, LEU 391, ALA 350	PHE 404	-	-
Anthocyanidins	cyanidin	-	LEU 387, LEU 391, ALA 350, LEU 525	PHE 404	-	GLU 353 (acceptor-acceptor)
Phytosterols	beta-sitosterol	-	LEU 354, TRP 383, ALA 350, LEU 525, LEU 346	-	-	-
	campesterol	-	TRP 383, ALA 350, LEU 525, LEU 346	-	-	-
	delta-7 avenasterol	-	TRP 383, ALA 350, LEU 525, LEU 346	-	-	-
	fucosterol	-	TRP 383, LEU 525, ALA 350, LEU 346	-	-	-
	stigmasterol	-	LEU 346, ALA 350, LEU 525, TRP 383	-	-	-
Gibberellin A19 Analogs	Analog 1	-	ALA 350, PHE 404, LEU 346	-	-	-
	Analog 2	LEU 346, MET 421, GLU 521	LEU 387, ALA 350, LEU 384, LEU 525	-	-	-
	Analog 3	-	ALA 350, LEU 346, PHE 404	-	-	-
	Analog 4	HIS 524	LEU 525, ALA 350, PHE 404, LEU 346	-	-	-

tabulated in Table 2 and 3. Estradiol (E2) interacts with the wild-type ER- α through H-bonding and hydrophobic interactions. The amino acid residues involved are HIS 524 (H-bonding), ILE 424, MET 388, LEU 391, LEU 387, ALA 350 (hydrophobic interactions), and PHE 404 (π - π stacking). Tamoxifen interacts with the wild-type ER- α through the same types of interactions which involved THR 347 (H-

bonding), ILE 424, MET 388, LEU 391, LEU 387, ALA 350, LEU 525 (hydrophobic interactions), PHE 404 (π - π stacking), and MET 421 (π -S). Unfavorable interaction involving amino acid residue GLU 353 (unfavorable donor-donor) is also present.

Shown in Table 3 are the different amino acid residues of the mutant ER- α (PDB ID: 5TLT) and the different ligands.

Table 3. Protein-ligand interactions between Estradiol, Tamoxifen, *Phaseolus vulgaris* L. compounds, Gibberellin A19 analogs, and the Mutant ER- α (PDB ID: 5TLT)

Compound Class	Compound	Hydrogen Bonding	Hydrophobic Interactions	π - π Stacking Interactions	Miscellaneous Interactions	Unfavorable Interactions
Endogenous Ligand	Estradiol	GLY 521, GLU 353	ILE 424, MET 388, LEU 387, PHE 404, ALA 350, LEU 391	PHE 404	-	-
SERM	Tamoxifen	-	LEU 346, LEU 387, ALA 350, LEU 387, LEU 346, GLY 420, MET 421	PHE 404	MET 421(π -S)	-
Flavanone	naringenin	MET 421, GLU 353, ARG 394	ILE 424, LEU 391, LEU 387, MET 421	PHE 404	-	-
	pinocembrin	MET 421	ALA 350, LEU 346, MET 421, ILE 424	-	-	-
Flavone	apigenin	GLU 353, LEU 387, ARG 394, GLY 521	ALA 350, LEU 391, LEU 387, LEU 384, ILE 424	PHE 404	-	-
	luteolin	GLU 353	ILE 424, MET 421, LEU 391, LEU 387, ALA 350	PHE 404	-	-
Isoflavone	daidzein	GLU 353	LEU 391, LEU 387, ALA 350, LEU 346, LEU 525	PHE 404	-	-
Flavononol	aromadendrin	PHE 425, LEU 387	MET 421, ILE 424, LEU 391, ALA 350, LEU 387	PHE 404	-	ARG 394 (donor-donor)
Anthocyanidins	cyanidin	ARG 394, GLU 353, LEU 346	LEU 391, LEU 387, ALA 350	PHE 404	-	-
Phytosterols	beta-sitosterol	GLU 353	MET 343, MET 388, LEU 384, LEU 387, LEU 391, ALA 350, PHE 404	-	-	-
	campesterol	-	MET 343, LEU 384, LEU 387, ALA 350, LEU 391, PHE 404, MET 388	-	-	-
	delta-7 avenasterol	-	LEU 384, ALA 350, LEU 387, MET 388, LEU 428, LEU 391, MET 421, HIS 524, ILE 424	-	-	-
	fucosterol	HIS 524	LEU 391, LEU 387, ALA 350, LEU 346, LEU 525, MET 528, MET 421, MET 343, HIS 524	-	-	-
	stigmasterol	-	VAL 418, HIS 524, MET 528, MET 343, MET 388, LEU 391, PHE 404, ALA 350, LEU 387	-	-	-
Gibberellin A19 Analogs	Analog 1	GLU 353	LEU 387, LEU 346, ALA 350	-	-	-
	Analog 2	GLY 521	LEU 346, LEU 387, ALA 350, LEU 525, LEU 384	-	-	-
	Analog 3	GLU 353	LEU 387, LEU 346, ALA 350	-	-	-
	Analog 4	MET 421, LEU 346	LEU 525, MET 528, ILE 424	-	MET 343 (pi-sulfur)	-

The mutant ER- α 5TLT was used as the representative for the different mutant receptors since the binding of the PVL ligands toward this receptor is the most favorable. Moreover, superimposition of the structures of the different mutant receptors, as shown in Figure 1, indicates that there were no significant differences in their structures and that the ligand binding pocket was preserved. Deviations in the structures of the mutant ER- α models were not within the vicinity of the ligand binding pocket.

For estradiol (E2), most of the interactions involved were hydrogen bonding and hydrophobic interactions. Amino acids GLU 353 and GLY 521 were involved in the hydrogen bonding interactions between estrogen and the wild-type and mutant ER- α . For hydrophobic interactions, the amino acids involved were ILE 424, MET 388, LEU 387, PHE 404, ALA 350, and LEU 391. The amino acid PHE 404 was also involved in π - π stacking interactions. Tamoxifen interacted with the ER- α by hydrogen bonding through the amino acid

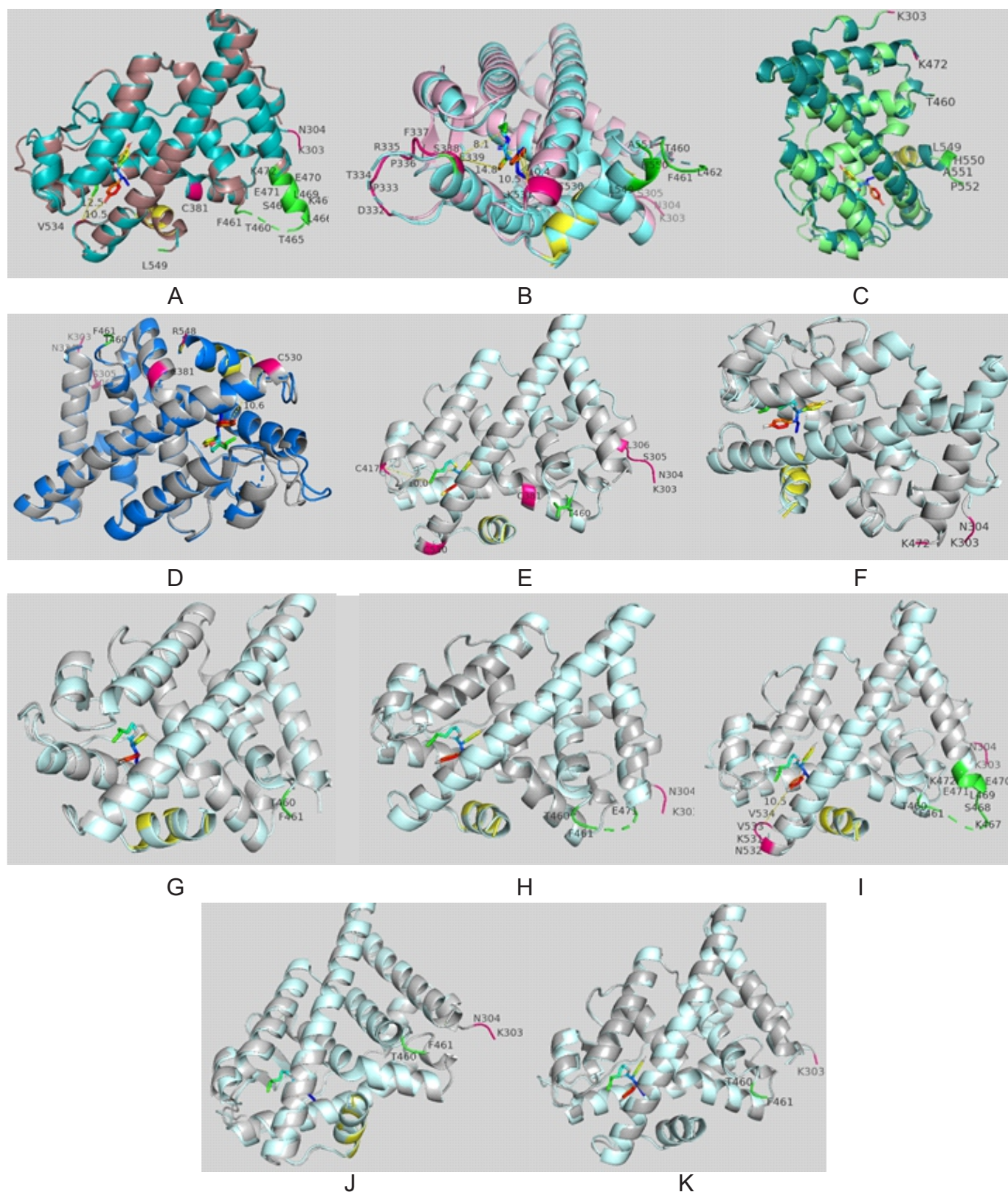


Figure 1. Superimposed structures of the mutant receptors with the ER- α PDB ID: 5TLT as the reference receptor. A) PDB ID: 3UUD, B) PDB ID: 4ZNS, C) PDB ID: 4ZNV, D) PDB ID: 5DXE, E) PDB ID: 5DXG, F) PDB ID: 5KCD, G) PDB ID: 5KD9, H) PDB ID: 5KCT, I) PDB ID: 5TM8, J) PDB ID: 5TN4, K) PDB ID: 5TN5. Labeled also are the amino acid residues that are different between the structures. The perturbation closest to the ligand-binding cleft can be seen in B which is 8 Å involving E338 residue. Shown also in rainbow is 7ED, an investigational SERM which is the co-crystallized ligand of the ER- α PDB ID: 5TLT. The structure highlighted in yellow is helix 12, an essential part of the 3D structure of the ER- α involved in the activation of the receptor into agonist form. The calculated RMSD values of the superimposed receptors with respect to 5TLT are less than 2 Å.

THR 347 in the wild-type ER- α whereas in the mutant receptor, this was absent. Hydrophobic interactions were also responsible for most interactions between tamoxifen and the estrogen receptors. Several amino acids were involved in the said hydrophobic interactions which were LEU 346, LEU 387, ALA 350, LEU 387, LEU 346, GLY 420, and MET 421. Like in the case of E2, PHE 404 was involved in π - π stacking interactions and a miscellaneous interaction involving MET 528 through pi-sulfur interaction is also present.

The PVL compounds interacted with the wild-type ER- α mostly through hydrophobic interactions which involved ALA 350, GLU 353, GLY 521, HIS 524, ILE 424, LEU 384, LEU 346, LEU 387, LEU 391, LEU 349, LEU 384, LEU 387, LEU 391, LEU 525, MET 343, MET 388, MET 421, MET 522, PHE 404, AND and TRP 383. π - π stacking interactions which involved PHE 404 is were also observed between the wild-type ER- α and all the flavonoids. Hydrogen bonding is also a type of interaction present between the flavonoids and the wild-type receptor which often involves the amino acid residues HIS 524, ARG 394, GLU 353, PHE 404, and GLY 521.

As with the case of the other compounds, most interactions present between gibberellin A19 analogs, and the estrogen receptors alpha were hydrogen bonding and hydrophobic interactions. These involved amino acids GLU 353 and GLY 521 for H-bonding whereas ALA 350, LEU 525, and LEU 384 were some of the amino acids involved in hydrophobic interactions.

In Table 4, the structures, molecular formula, molecular weight, and the synthesizability scores of the analogs are shown. Presented in Table 5 are their absorption, distribution, metabolism, and excretion profiles are presented. 'Pgp-inh' stands for p-glycoprotein inhibitor, 'pPgp-sub' for p-glycoprotein substrate, 'HIA' for human intestinal absorption, 'F' for oral bioavailability, 'BBB' for blood -brain barrier, 'PPB' for plasma protein bound, and 'CL' for clearance. The values are given as probability. For the absorption and distribution, the values are given as the probabilities of being a pPgp-inh, being pPgp-sub, and being poorly absorbed; probability of having an oral bioavailability of less than 20% or 30%, probability of not penetrating the blood -brain barrier, and the percentage of being protein bound. Hence, for Pgp-inh, Pgp, sub, HIA, and F, values within the range of 0-0.3 are desirable while for BBB, a value of 0.7 to 1.0 is desirable in the context of this study. For PPB, values less than or equal to 90% are desirable and for clearance (CL), values greater bound within 5 are preferred. In Table 6, the metabolism profile of the analogs is presented. Given in each column are the family

of cytochrome enzymes and the probabilities of being a substrate or inhibitor. As much as possible, it is desirable that the probability values are from 0 to 0.7. For the toxicity profile given in Table 7, the columns represent human ether-a-go-go related gene toxicity (hERG), human hepatotoxicity (H-HT), drug-induced liver injury (DILI), Ames toxicity, rat oral toxicity (ROA), carcinogenicity, respiratory toxicity, probability to bind to androgen receptor (NR-AR), probability to bind to androgen receptor ligand-binding domain (NR-AR-LBD), probability to act as aromatase inhibitor (NR-aromatase), probability of binding to estrogen receptor (NR-ER), and estrogen receptor ligand-binding domain (NR-ER-LBD). For the context of this study, values ranging from 0 to 0.3 are desirable for hERG, H-HT, DILI, Ames, ROA, carcinogenicity, and respiratory columns. For the rest of the columns, values higher than 0.3 are preferred [34].

Discussion

ER- α has been established to be involved in cancer progression and metastases. One mechanism is through mutation in the ligand binding domain of the receptor [8,46,47]. The most common and most invasive of which is the Y537S type [48,49]. In this study, the crystal structures of the wild-type ER- α (PDB ID: 1A52) and the 12 mutant estrogen receptors alpha of the Y537S type (PDB ID: 3UUD, 4ZNS, 4ZNV, 5DXE, 5DXG, 5KCD, 5KCT, 5KD9, 5TLT, 5TM8, 5TN4, and 5TN5) were used in *in silico* screening of PVL compounds as potential anticancer agents. The presence of Y537S mutation makes the receptor constitutively active, independent of the native ligand estradiol. In the agonist conformation, the helix 12 (H12) rests across H3 and H11 to form an indentation which accommodates co-regulator binding [50]. This structural feature is seen in Figure 2. Note that 1ERE (wild-type and in agonist conformation) was utilized instead of 1A52 (wild-type and in agonist conformation) to compare the structural features since the helix 12 of the former is more exemplified than the former. Hence, superimposing the structures make the visualization easier.

The Y537S mutation also results to in the replacement of Y537-N348 interaction with S537-D351 hydrogen bonding which optimizes the H11-H12 loop in the agonist conformation allowing the coactivators to be recruited to the AF2 cleft. The stabilization of the agonist conformation also reduces the affinity of tamoxifen towards the receptor [51]. This is evident when tamoxifen was docked in the mutant receptors 3UUD, 5DXE, and 5DXG as compared to the wild-type receptor 1A52. In these mutant receptors, estradiol is the co-crystallized ligand, hence the receptor is in the agonist

Table 4. Swiss ADME results of the Gibberellin A19 analogs

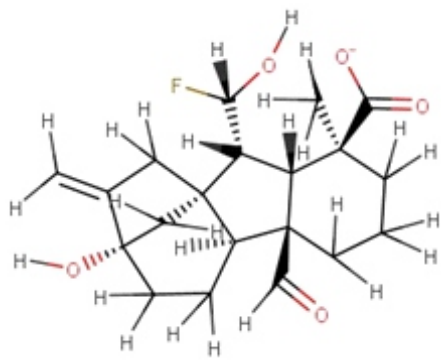
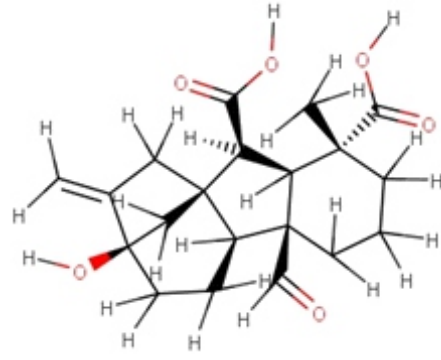
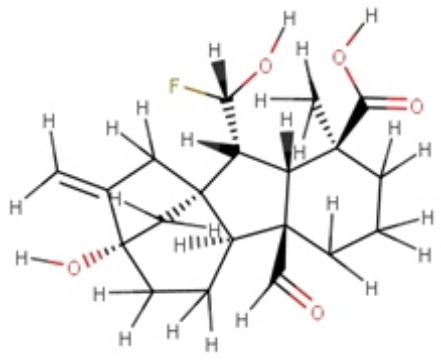
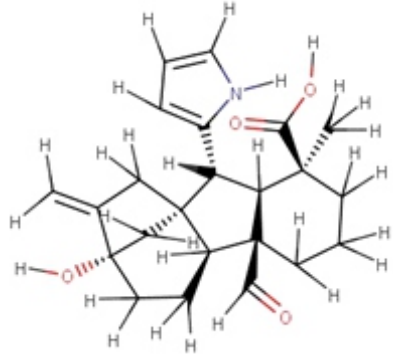
Molecule	Formula	MW (g/mol)	Synthetic Accessibility	Structure
Analog 1	$C_{20}H_{26}FO_5$	365.42	5.54	
Analog 2	$C_{20}H_{26}O_6$	362.42	5.41	
Analog 3	$C_{20}H_{25}FO_5$	366.42	5.57	
Analog 4	$C_{23}H_{29}NO_4$	383.48	5.4	

Table 5. Absorption, distribution, and excretion profile of Gibberellin A19 analogs obtained from the ADMETlab2.0 web server

Compound	Absorption					Distribution		Excretion
	Pgp-inh	Pgp-sub	HIA	F(20%)	F(30%)	BBB	PPB	CL
Analog 1	0	0.964	0.015	0.89	0.556	0.836	52.86%	2.224
Analog 2	0.001	0.01	0.04	0.952	0.101	0.828	58.67%	1.005
Analog 3	0	0.964	0.015	0.89	0.556	0.836	52.86%	2.224
Analog 4	0	0.784	0.005	0.133	0.014	0.912	49.19%	2.496

Table 6. Metabolism profile of Gibberellin A19 analogs obtained from the ADMETlab2.0 web server

Compound	Metabolism									
	CYP1A2-inh	CYP1A2-sub	CYP2C19-inh	CYP2C19-sub	CYP2C9-inh	CYP2C9-sub	CYP2D6-inh	CYP2D6-sub	CYP3A4-inh	CYP3A4-sub
Analog 1	0.002	0.925	0.007	0.239	0.005	0.053	0.004	0.069	0.758	0.075
Analog 2	0.001	0.754	0.008	0.083	0.01	0.119	0.007	0.083	0.242	0.013
Analog 3	0.002	0.925	0.007	0.239	0.005	0.053	0.004	0.069	0.758	0.075
Analog 4	0.004	0.889	0.014	0.128	0.012	0.097	0.013	0.084	0.844	0.12

Table 7. Toxicity profile of Gibberellin A19 analogs obtained from the ADMETlab2.0 web server

Compound	Toxicity Profile										
	hERG	H-HT	DILI	Ames	ROA	Carcinogenicity	Respiratory	NR-AR	NR-Aromatase	NR-ER	NR-ER-LBD
Analog 1	0.022	0.698	0.037	0.067	0.791	0.665	0.985	0.949	0.844	0.533	0.008
Analog 2	0.01	0.183	0.054	0.02	0.101	0.14	0.839	0.882	0.619	0.387	0.01
Analog 3	0.022	0.698	0.037	0.067	0.791	0.665	0.985	0.949	0.844	0.533	0.008
Analog 4	0.035	0.665	0.033	0.03	0.943	0.587	0.985	0.968	0.919	0.899	0.102

conformation. However, the binding affinities of tamoxifen towards 3UUD, 5DXE, and 5DXG have significantly decreased as compared to its binding affinity towards 1A52 as shown in Table 1. This is also evident in Figure 3A, wherein the structure of the wild-type ER- α bound to 4-hydroxytamoxifen (PDB ID: 3ERT), a SERM, was superimposed to the structure of the mutant ER- α bound to 7ED (PDB ID: 5TLT), an investigational SERM. Note that 3ERT was utilized for visualization of the antagonist conformation of the wild-type receptor and not in the molecular docking experiment. Highlighted in the structures is the helix 12 of the receptors. The image shows that the helix 12 (green and violet), which is crucial in the agonist-antagonist switching of the receptor, does not overlap. If both structures bind SERM, they should be in the antagonist conformation but that is not the case in 5TLT. Moreover, to exemplify the stabilization of the agonistic form in mutant receptors, the structure of wild-type ER- α bound to estrogen (PDB ID: 1ERE) is overlaid to the structure of mutant ER- α 5TLT in Figure 3B. As shown, the helix 12 (red

and green) of the structures overlapped, indicating that 5TLT is in agonistic conformation despite being bound to 7ED.

As previously mentioned, 5TLT was used as the mutant receptor reference. Figure 3 shows the image of the overlapped structures. In all the mutant receptors structures, the helix 12 overlapped. Moreover, the differences in the amino acid residues in the mutant estrogen receptors alpha are also labeled. The RMSD values between the 5TLT structure and the other mutant receptors are below 2 Å. The RMSD value indicates the average deviation between corresponding atoms of two proteins and that the smaller the value, the higher is the similarity [52]. Moreover, the deviations in the amino acid residues in the structure do not directly interact within the ligand binding pocket. The closest possible nonbonding interaction to the deviation in amino acid residue is 8.1 Å as shown in Figure 1. This distance is large even for an effective hydrophobic interaction (5.5 Å) or any unfavorable interactions (5.6 Å) [33]. Additionally, the ligand

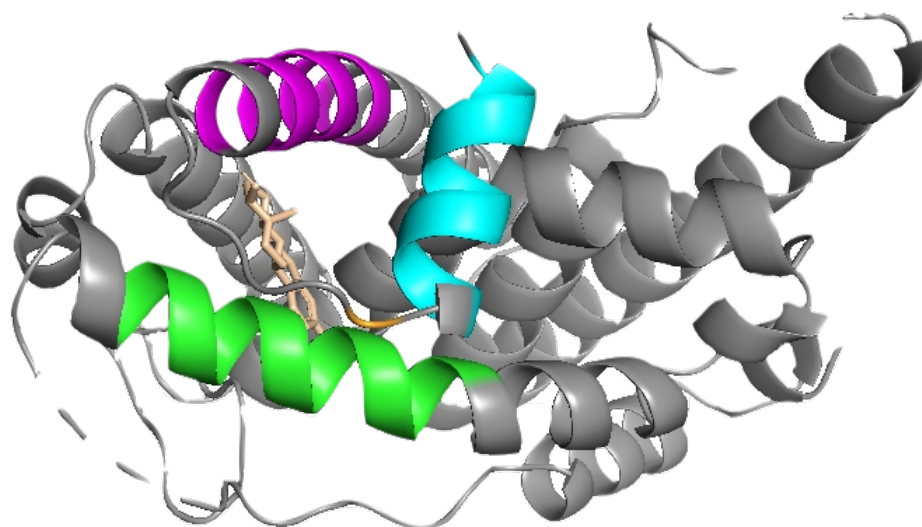


Figure 2. Structure of wild-type ER- α (PDB ID: 1ERE). Highlighted in cyan is helix 12, in purple is helix 11 and in green is helix 3. The co-crystallized ligand E2 is also shown in cream. Shown here is helix 12 resting over helices 11 and 3 which stabilizes the agonist conformation.

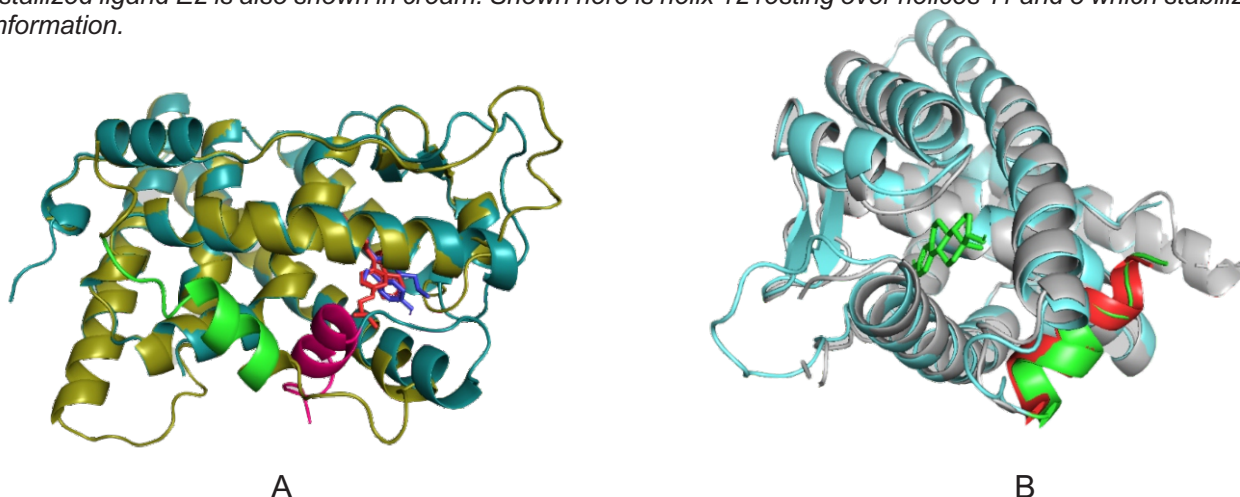


Figure 3. Superimposed structures of the A) wild-type ER- α (PDB ID: 3ERT) (olive green) bound to tamoxifen and mutant ER- α (light blue) bound to 7ED (PDB ID: 5TLT) and B) Image of superimposed mutant ER- α 5TLT (light blue) and wild-type ER- α 1ERE (gray) bound to estradiol.

binding pocket is formed by parts of helices 3, 6, 8, and 11, and 12 and S1/S2 hairpin involving the amino acid residues MET 342 to LEU 354, TRP 383 to ARG 394, VAL 418 to LEU 428 and MET 517 to MET 528, LEU 539 to HIS 547 and LEU 402 to LEU 410 [53,54] and the deviations in amino acid residues are not found within these helices. Since a rigid receptor-flexible ligand type of molecular docking was conducted, the differences in the binding affinity values across the different mutant receptors can be attributed mainly to the structures of the ligands and the scoring function of Autodock Vina [55].

Table 2 and Table 3 show that there are similarities in the interacting amino acids between the ER- α , the endogenous

ligand estradiol, the SERM tamoxifen, as well as the PVL ligands. This, in addition to the favorable binding energies across the different mutant receptors and the wild-type receptor presented in suggests the ability of PVL compounds to act either as agonist or antagonist [50,56]. The similarity in structure between E2 and flavonoids allows the latter to act as ER- α modulators. The distance between the hydroxyl groups and the phytoestrogens is almost the same, at 14.5 Å [57]. Moreover, the 4'-OH group of the flavonoids binds in the same way as the ring A of E2, forming hydrogen bonds with the residues GLU 353 and ARG 394 [58]. Lee and Barron (2017) mentioned that THR 347 and HIS 524 are also involved in hydrogen bonding and overall, these interactions help in

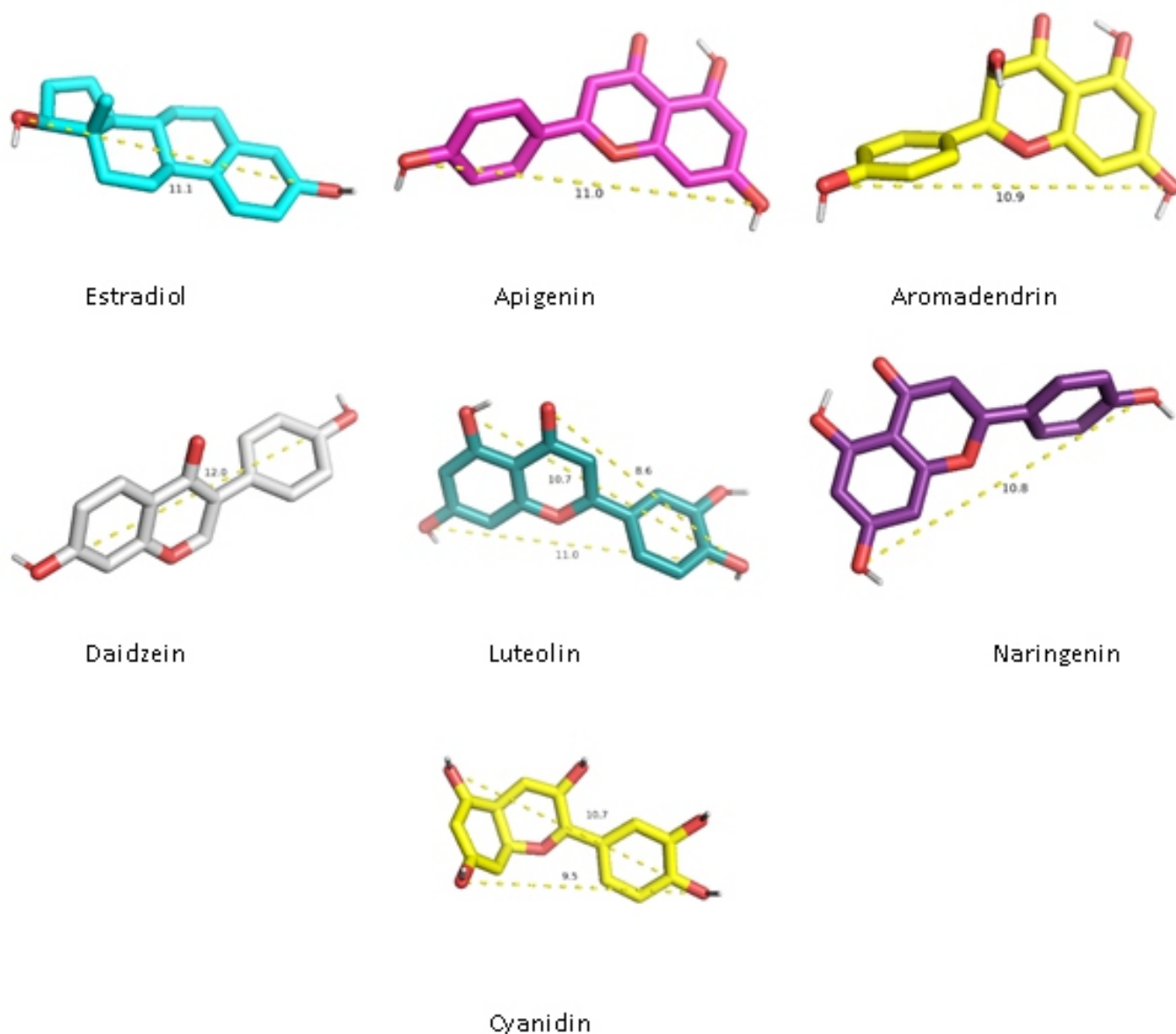


Figure 4. Distances of the hydroxyl groups present in the structure of E2 and some of the ligands.

attaching the ligand in the ligand binding pocket [59]. The distance obtained in E2 is 11.1 Å and the rest of the compounds have the distances of their hydroxyl groups (4'-OH phenyl hydroxyl and OH at position 6) around this value (9-12 Å) (Figure 4).

Aside from hydrogen bonding, hydrophobic forces make up majority of the protein-ligand interactions. This occurs via the phenyl group, like the ring A of E2. The presence of the aromatic ring is also a characteristic of a good ligand for ER alpha [59]. and this is present also in the structures of PVL flavonoids. In the interaction analysis of synthesized coumarin derivatives conducted by Shylaja *et al.* (2021) [26], involving

the same receptors (wild-type and mutant receptors), PHE 404 via π - π stacking interactions is present when ER alpha interacts with an agonist. This type of interaction involving PHE 404 can be seen in the flavonoids found in PVL compounds. Moreover, in the same study, the other amino acid residues involved in hydrophobic interactions involves HIS 524, ILE 424, LEU 428, LEU 391, and LEU 346. Interactions between these amino acid residues are essential to stabilize H3 and H11 in agonist conformation since these hydrophobic interactions hold H11, H8, H6, beta-sheet, and H3 together. In Table 2 and Table 3, these amino acid residues are also the recurring amino acids that are present in the hydrophobic interactions between the flavonoids of PVL and the ER alpha.

These same interactions are also present in the phytosterols. This strengthens the idea that the flavonoids, which are also phytoestrogens, can bind and modulate ER- α activity in both wild-type and mutant receptors.

In the macromolecule-ligand-complex based pharmacophore modeling conducted by Shylaja *et al.* (2021) using the same mutant receptors, they found that the pharmacophoric features of good ligands for these ER- α receptors possess two aromatic rings, a hydrogen bond acceptor or donor and hydrophobic features [26]. All the flavonoids found to be present in PVL possess these characteristics. The structures of flavonoids found in PVL contain two aromatic ring moieties, possible hydrogen bond donors and acceptors, and hydrophobic moieties. This further explains the favorable interactions and binding affinities observed with flavonoids when docked in the ER- α receptors. The results of the current study are also in coherence with the results obtained by D'Arrigo *et al.* (2021) [60] which involved apigenin, genistein, luteolin, naringenin, quercetin, and resveratrol [60]. In their study, these six flavonoids were docked in nuclear receptors including ER- α and the compounds showed good docking scores and complementarity with the ER- α and ER- β . Their study also showed that E2 obtained the most favorable binding score.

In an *ex vivo* experiment conducted by Chen and Chien (2019) [61] which utilized breast cancer cells from breast cancer patients, and involved genistein, resveratrol, and quercetin, it was found that the phytoestrogens inhibit human breast cancer viability [61]. Moreover, proteins involved in the apoptotic pathway and estrogen receptor beta were increased in cells that were treated with phytoestrogens. These results suggest that the flavonoids have potential anti-breast cancer capabilities. Certainly, the ability of flavonoids to act against breast cancer has been widely studied in literature [23,60-62].

While the agonistic interactions between the ER- α (wild-type and mutant) are present, Tables 4 and 5 shows that the amino acid residues involved in the interaction of tamoxifen with the ER- α receptors are also like that of the interaction between PVL ligands and the receptors. This similarity in amino acid residues suggests that the PVL compounds can also interact with ER- α in an antagonistic manner. Shylaja *et al.* (2021) identified four hydrophobic holes that are present within the ligand binding pockets which involved LEU 384: LEU 387: LEU 391 (hydrophobic hole 1), PHE 404: ILE 424 (hole 2), LEU 349: ALA 350 (hole 3), and, MET 343: LEU 346, LEU 525 (hole 4) [26]. All these amino acids are present in the interaction between the flavonoids in PVL as well as the

phytosterols. These protein-ligand interactions indicate that the flavonoids, lignan, and stilbene (phytoestrogens) can also act as an antagonist. The agonistic and antagonistic activities of these phytoestrogens are beneficial when there is a deficiency in estrogen levels or overexpression of estrogen receptors [57].

Another class of compound found in PVL are the phytosterols. Their core structure is cholesterol which is also a precursor for the biosynthesis of E2 in the human body [65]. Plant sterols are known to exhibit anticancer properties [66]. A phytosterol, beta-sitosterol, is also considered as a phytoestrogen and has been shown *in vitro* to competitively bind to ER- α [65]. As seen in Table 2 and Table 3, the phytosterols present in PVL bind favorably, to some extent, to both the wild-type and mutant estrogen receptors alpha. This suggests a possible competitive binding or even an agonistic binding. This idea is strengthened by the fact that the amino acid residues involved in the interaction between E2 and the ER- α receptors as well as between the phytosterols and the receptors, both the wild-type and the mutant receptor have some degree of similarities as shown in Table 2 and 3. As previously mentioned, there were instances wherein the binding of phytosterols toward the mutant receptors is unfavorable. It should be noted that while there is no significant difference between the structures of the mutant receptors used in the study (RMSD < 6.0 Å), it is not equal to zero. This suggests that these fine differences (which may include distances among the different residues and the spatial orientation) may have contributed to these deviations [33]. Moreover, an examination of the structures of phytosterols reveals that there are various rotatable bonds present in the side chains which may orient itself in different ways towards the receptor which may lead to less favorable binding energies. In a quantitative structure activity relationship study conducted by Gao *et al.* (1999) involving estrogen receptor and its ligands, they found that the receptor has limited tolerance to steric effects at the number 16 position of estradiol [66]. If the structure of the phytosterols and E2 are compared, this position is in proximity with to the position 17 where the side chains of the phytosterols are situated. This supports the idea that steric clashes from the phytosterols result to in unfavorable binding. Moreover, in the Autodock Vina Program, the presence of steric effects and steric and hydrophobic interactions, based on the weighted scoring function utilized, results to in a less negative binding score [32].

Of all the analogs generated, only those of the gibberellin A19 bioisosteres were found to have favorable interactions across the different ER- α models and were found to have

good ADMETox profile from the MolOpt web server. Analogs of gibberellin A19 were summarized in Table 4.

These analogs have comparable binding affinity across the different estrogen receptors alpha with respect to E2 and tamoxifen. The structures of the analogs are shown in Table 4, whereas their interactions with the amino acid residues of ER are summarized in Table 2 and Table 3. As seen in the table, the amino acid residues have some similarities with the amino acid residues interacting with both E2 and tamoxifen. Specifically, ARG 394, GLU 353, and HIS 524 are all involved in hydrogen-bonding whereas amino acids like LEU 540, LEU 525, ALA 350, LEU 346, PHE 404, and MET 388 are involved in hydrophobic interactions. While there are similarities, it should be noted that the structures contain an acidic moiety which becomes protonated once it reaches the target site of the microtumor environment. MarvinSketch v21.20.0 reveal that the acidic moieties have a pKa of about 4.6. Hence, it is most likely to be in the deprotonated state at the target site since the pH of the microtumor environment is around 6.7 [69]. This poses a problem since the target, ER- α , is mostly situated inside the cell, particularly the nucleus. It will be difficult for the analogs to penetrate the cell since they will be in their charged form, unless there are cellular transporters which can recognize these analogs and transport them inside the cell [70]. The presence or absence of transporters for the analogs is beyond the scope of this paper.

ADMETlab 2.0 results of the gibberellin A19 analogs presented in Table 7 reveal that these analogs are predicted to have high intestinal absorption but low oral bioavailability. They all passed the Lipinski's rule of 5 drug-likeness rules. Also, as predicted in their structure (highly polar because of the carboxylic acid moiety, fluorine, hydroxyl groups, carbonyl carbon, negative charges), they cannot cross the blood-brain barrier, suggesting that they may not cause neurologic side effects, unlike other anticancer drugs which cause central or peripheral nervous system complications [71]. All of them have less interactions with the CYP enzymes indicating that the first pass metabolism or drug interactions may be lessened or avoided. First pass metabolism and pharmacokinetic drug interactions may lessen the drug in the site of action, making it less effective or may heighten toxic effects [72-74]. The Swiss ADME web server also predicted that there will be some difficulty in synthesizing these compounds since they obtained a score of a little over 5. In the Swiss ADME training data sets, a score of 10 indicates a very difficult synthesizability whereas a score of 1 indicates otherwise [28].

Based on the ADMETlab2.0 results presented in Table 5, the gibberellin A19 analogs are most likely to be substrates of p-glycoprotein. This will become a problem later because p-glycoproteins are part of efflux mechanisms of cancer cells which lead to treatment resistance [75]. Since the PPB (plasma protein binding) percentage is less than 90%, they are predicted not to be highly protein bound. This is a good characteristic of the analogs because highly protein-bound drugs tend to have a low therapeutic index. For the excretion profile, the clearance probabilities are given in Table 5. Since the value is less than 5, they are predicted to exhibit low clearance. This has implications in the frequency dosing of the compounds since frequent dosing with low clearance of the drugs can induce toxic side-effects. This agrees with their metabolism profile (Table 6) since they interact less with the CYP enzymes except in some instances such as that of analog 1 and its protonated analog 3 which are predicted to be substrates of CYP1A2. These two compounds are also predicted to be CYP3A4 inhibitors. This prediction leads to the idea that they tend to undergo first pass metabolism and the amount of the intact compound which goes to the target site is decreased [72].

Table 7 shows that the compounds show hepatotoxicity (H-HT probability score > 0.3) and moderate carcinogenicity except for Analog 2 (probability score is 0.14 < 0.3). They are predicted to test negative in the Ames test and all of them are predicted to be orally toxic when administered to rats except for analog 2. Moreover, all of them are predicted to be highly toxic in to the respiratory system (probability score > 0.3). They are, however, not cardiotoxic (hERG probability score < 0.3). It is noteworthy that they are predicted to bind to the androgen receptors, aromatase enzyme, and estrogen receptor (probability scores > 0.3). As previously discussed, these receptors and enzymes play a crucial role in the pathogenesis of breast cancer and the ability of these compounds to be act as ligands to these proteins may suggest that they can act as either agonist, antagonist, or receptor modulators. Nevertheless, as predicted in their structures, the compounds will have difficulty reaching the ligand-binding domain of the estrogen receptor as supported by the low probability in the NR-ER-LBD column of Table 7 [24]. These constellations of results suggest that further optimization of the analogs should be done to improve their ADMET profiles.

Conclusion

Phaseolus vulgaris L. (fam. Fabaceae) contains compounds which belong to the group of phytoestrogens, phytosterols,

and plant hormones. The phytoestrogens, phytosterols, and plant hormone analogs show favorable binding affinity towards the wild-type ER- α and the mutant receptors of Y537S type. The most common interactions between E2, tamoxifen, and the PVL ligands were found to be hydrogen-bonding, hydrophobic, and π - π stacking interactions. The similarities in the amino acid residues that interact with E2, tamoxifen, and the PVL ligands in addition to the favorable binding energies, suggest that these compounds are possible candidates against ER-positive breast cancer. The compounds and analogs presented in this work can be further optimized to obtain compounds which can outperform E2 in terms of binding and activity towards ER- α , especially the mutant types. Moreover, molecular dynamics simulation, and *in vitro* tests can be conducted to surpass the limitations of the current study.

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Supplementary Table 1. Grid Box Optimization Parameters

Estrogen Receptors (PDB ID)	Co-crystallized ligands	x-coordinates	y-coordinates	z-coordinates	RMSD Values (Å)
1A52	E2	106.253	17.463	97.957	0.560
3UUD	E2	21.210	4.511	4.837	0.761
4ZNS	OFB	-19.390	-26.746	-6.127	0.512
4ZNV	4Q7	2.588	11.770	-20.759	0.407
5DXE	E2	25.359	-2.078	5.790	0.690
5DXG	E2	25.680	-1.720	5.847	0.866
5KCD	OB2	25.052	14.052	7.841	0.464
5KCT	OB6	-19.513	-28.461	-5.795	1.219
5KD9	OBT	-18.961	-25.460	-5.244	0.953
5TLT	7ED	18.585	12.920	3.675	1.217
5TM8	K6	-16.218	2.584	49.289	1.778
5TN4	7FZ	19.202	-1.871	2.862	0.801
5TN5	7G0	-17.011	-26.331	-2.624	1.268

Supplementary Table 2. List of secondary metabolites found in *Phaseolus Vulgaris* L. (fabaceae)

Count	Compounds	Aglycone	Plant Part Used	Country of Origin	Variety	References
1	(+)-catechin glucoside	(+)-catechin	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
2	(+)-piscidic acid	(+)-piscidic acid	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
3	2,6-dihydroxybenzoic acid	2,6-dihydroxybenzoic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
4	3-hydroxybenzoic acid	3-hydroxybenzoic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
5	4-hydroxybenzoic acid	4-hydroxybenzoic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
6	apigenin 6,8-di-c-glucoside	Apigenin	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
7	aromadendrin glucoside	Aromadendrin	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
8	benzoic acid	benzoic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
9	beta-sitosterol	beta-sitosterol	beans	Mexico	San Luis	Ramírez-Jiménez <i>et al.</i> (2015)
10	biochanin a 7-o-[b-d-apiofuranosyl-(1-->5)-b-d-apiofuranosyl-(1-->6)-b-d-glucopyranoside]	Biochanin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
11	caffeic acid 4-o-glucoside	caffeic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
12	β-campesterol	Campesterol	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i> (2019)
13	carthamidin glucoside	Carthamidin	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
14	chlorogenic acid	chlorogenic acid	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
15	chrysoeriol 7-glucoside	Chrysoreriol	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
16	cinnamic acid	cinnamic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
17	cis ferulic acid	cis ferulic acid	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
18	citric acid	citric acid	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)

Supplementary Table 2. *List of secondary metabolites found in Phaseolus vulgaris L. (Fabaceae)(continuation)*

Count	Compounds	Aglycone	Plant Part Used	Country of Origin	Variety	References
19	coumesterol	Coumesterol	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
20	cyanidin	Cyanidin	beans	Brazil	BR1-XODÓ	Barreto <i>et al.</i> (2021)
21	6"-o-malonyldaidzin	Daidzein	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
22	dalbinol	Dalbinol	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
23	dalpanin	Dalpanin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
24	delphinidin 3,5-o-diglucoside	Delphinidin	beans	Italy	genotype MG53	Madrera and Valles (2020)
25	delta-7-avenasterol	delta-7-avenasterol	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i> (2019)
26	eriodictyol	Eriodictyol	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
27	escopoletin	Escopoletin	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
28	hydroxybenzyl-malic acid (eucomic acid)	eucomic acid	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
29	formononetin 7-o-glucoside (ononin)	Formononetin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
30	fucosterol	Fucosterol	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i> (2019)
31	gallic acid 4-o-glucoside	gallic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
32	gallic acid ethyl ester	gallic acid ethyl ester	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
33	6"-o-malonylgenistin	Genistein	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
34	genistin	Genistin	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
35	gibberellin a 19	gibberellin a 19	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
36	gibberellin a25	gibberellin a25	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
37	gibberellin a38	gibberellin a38	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
38	6"-o-malonylglycitin	Glycitein	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
39	heliangin	Heliangin	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
40	hesperidin	Hesperitin	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i> (2019)
41	hydroxyeucomic acid	hydroxyeucomic acid	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
42	hydroxymethoxycinnamic acid	hydroxymethoxycinnamic acid	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
43	isoferulic acid	isoferulic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
44	isolariciresinol 9-o-b-d - glucopyranoside i	Isolariciresinol	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
45	isorhamnetin 3-glucuronide	Isorhamnetin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
46	isosakuranetin 7-rutinoside	Isosakuranetin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
47	kaempferol-rutinoside	Kaempferol	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
48	kutkoside	kutkoside aglycone	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
49	luteolin 7-o-rutinoside	Luteolin	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
50	malvidin 3-glucoside	Malvidin	beans	Brazil	BR1-XODÓ	Barreto <i>et al.</i> (2021)
51	myricetin	Myricetin	beans	Italy	genotype MG76	Madrera and Valles (2020)

Supplementary Table 2. *List of secondary metabolites found in Phaseolus vulgaris L. (Fabaceae)(continuation)*

Count	Compounds	Aglycone	Plant Part Used	Country of Origin	Variety	References
52	naringenin	Naringenin	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
53	naringenin 7-methyl ether	naringenin 7-methyl ether	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
54	naringin	Naringin	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
55	pelargonidin	Pelargonidin	beans	Brazil	BR1-XODÓ	Barreto <i>et al.</i> (2021)
56	peonidin	Peonidin	beans	Brazil	BR1-XODÓ	Barreto <i>et al.</i> (2021)
57	petunidin 3,5-o-diglucoside	Petunidin	beans	Italy	genotype MG55	Madrera and Valles (2020)
58	phaseolin	Phaseolin	beans	Mexico		García-Cordero <i>et al.</i> (2021)
59	phaseollinisoflavan	Phaseollinisoflavan	beans	Colombia	Cargamanto Blanco and ICA Quimbaya	Durango <i>et al.</i> (2002)
60	p-hydroxyphenyl acetic acid	p-hydroxyphenyl acetic acid	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
61	pinocembrin	Pinocembrin	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
62	procyanidin dimer b1	procyanidin dimer b1	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
63	procyanidin dimer b2	procyanidin dimer b2	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
64	procyanidin trimer c1	procyanidin trimer c1	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
65	procyanidin trimer c2	procyanidin trimer c2	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
66	prodelphinidin b	prodelphinidin b	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
67	protocatechuic acid 4-o-glucoside	protocatechuic acid	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
68	isoquercitrin	Quercetin	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
69	resveratrol glucoside	Resveratrol	beans	South Africa	Mongetes del Ganxet	Llorach <i>et al.</i> (2019)
70	rhamnetin	Rhamnetin	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
71	salicylic acid	salicylic acid	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
72	sinapic acid	sinapic acid	beans	Italy	genotype MG52	Madrera and Valles (2020)
73	soyasapogenol a	soyasapogenol A	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
74	soysaponin ag	oyasapogenol B	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i> (2019)
75	soyasapogenol d	soyasapogenol D	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
76	soyasapogenol e	soyasapogenol E	beans	Mexico	Pinto Durango bean cultivar	Herrera <i>et al.</i> (2019)
77	phaseoside i	soyasapogenol A	beans	Mexico	Dalia	Mendoza-Sánchez <i>et al.</i>
78	stigmasterol	Stigmasterol	beans	Mexico	San Luis	Ramírez-Jiménez <i>et al.</i> (2015)
79	taxifolin 3-o-rhamnoside	Taxifolin	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)
80	trans p-coumaroyl aldaric acid	trans p-coumaric acid	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
81	trans ferulic acid	trans ferulic acid	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
82	tryptophol	Tryptophol	beans	Spain	Canellini and Pinta	Aguilera <i>et al.</i> (2011)
83	vanillic acid b-glucoside	vanillic acid	Pods	Spain	Perona, Helda, Strike	Abu-Reidah <i>et al.</i> (2012)

Supplementary Table 3. Compounds from *Phaseolus vulgaris* L. (Fabaceae) which exhibited favorable oral bioavailability, human intestinal absorption, drug-likeness, and toxicity

Count	Compounds	HIA	F(20%)	F(30%)	Carcinogenicity	Non Genotoxic ^b Carcinogenicity	Genotoxic/ Carcinogenicity/ Mutagenicity	QED	Lipinski	ERA ^c as target
1	(+)-catechin ^a	0.035	0.998	1	0.185	0	0	0.51	Accepted	NP
2	(+)-piscidic acid ^a	0.132	0.042	0.089	0.007	0	0	0.465	Accepted	Y
3	2,6-dihydroxybenzoic acid	0.079	0.579	0.993	0.152	0	0	0.559	Accepted	N
4	3-hydroxybenzoic acid	0.01	0.017	0.571	0.026	0	0	0.61	Accepted	N
5	3-hydroxybenzoic acid	0.01	0.01	0.308	0.05	0	0	0.61	Accepted	N
6	apigenin	0.015	0.995	0.999	0.277	0	0	0.632	Accepted	Y
7	aromadendrin ^a	0.012	0.715	0.099	0.039	0	0	0.634	Accepted	NP
8	benzoic acid	0.013	0.003	0.011	0.026	0	0	0.611	Accepted	N
9	beta-sitosterol ^a	0.002	0.889	0.054	0.12	0	0	0.436	Accepted	Y
10	biochanin ^a	0.005	0.008	0.795	0.221	0	0	0.756	Accepted	N
11	campesterol ^a	0.002	0.942	0.059	0.148	0	0	0.47	Accepted	Y
12	Carthamidin	0.031	0.957	0.996	0.581	0	0	0.599	Accepted	Y
13	Chrysoeriol	0.032	0.95	0.998	0.083	0	0	0.672	Accepted	Y
14	citric acid	0.398	0.008	0.004	0.009	0	0	0.427	Accepted	N
15	Cyanidin	0.028	0.997	0.998	0.069	0	0	0.347	Accepted	N
16	Daidzein	0.008	0.23	0.856	0.617	0	0	0.7	Accepted	Y
17	Dalpanin	0.01	0.786	0.838	0.169	0	0	0.639	Accepted	N
18	delphinidin	0.054	0.999	0.999	0.04	0	0	0.301	Accepted	N
19	delta_7_avenasterol ^a	0.005	0.99	0.857	0.008	0	0	0.454	Accepted	Y
20	eriodictyol ^a	0.01	0.794	0.999	0.509	0	0	0.599	Accepted	Y
21	formononetin	0.004	0.006	0.896	0.488	0	0	0.775	Accepted	Y
22	fucosterol ^a	0.003	0.987	0.253	0.157	0	0	0.454	Accepted	Y
23	gallic acid	0.085	0.964	0.995	0.024	0	0	0.46	Accepted	N
24	gallic acid ethyl ester	0.013	0.794	0.961	0.035	0	0	0.487	Accepted	N
25	Genistein	0.01	0.863	0.992	0.316	0	0	0.632	Accepted	Y
26	gibberellin A19 ^a	0.016	0.611	0.048	0.376	0	0	0.524	Accepted	N
27	gibberellin A25 ^a	0.023	0.947	0.038	0.148	0	0	0.665	Accepted	N
28	gibberellin A38 ^a	0.014	0.943	0.265	0.881	0	0	0.482	Accepted	N
29	glycitein	0.005	0.008	0.199	0.393	0	0	0.756	Accepted	N
30	hesperitin ^a	0.007	0.009	0.974	0.522	0	0	0.789	Accepted	Y
31	isolariciresinol ^a	0.054	0.203	0.012	0.06	0	0	0.651	Accepted	Y
32	Isorhamnetin	0.024	0.03	0.978	0.047	0	0	0.572	Accepted	N
33	isosakuranetin ^a	0.005	0.003	0.905	0.674	0	0	0.887	Accepted	Y
34	Kaempferol	0.008	0.856	0.993	0.097	0	0	0.546	Accepted	Y
35	luteolin	0.047	0.998	1	0.095	0	0	0.511	Accepted	Y
36	malvidin	0.038	0.046	0.731	0.032	0	0	0.545	Accepted	N

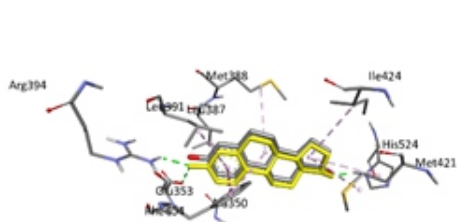
Supplementary Table 3. Compounds from *Phaseolus vulgaris* L. (Fabaceae) which exhibited favorable oral bioavailability, human intestinal absorption, drug-likeness, and toxicity (continuation)

Count	Compounds	HIA	F(20%)	F(30%)	Carcinogenicity	Non Genotoxic ^b Carcinogenicity	Genotoxic/ Carcinogenicity/ Mutagenicity	QED	Lipinski	ERA ^c as target
37	methyl 2-hydroxy-4-methoxy-6-methylbenzoate	0.012	0.006	0.274	0.032	0	0	0.728	Accepted	N
38	myricetin	0.035	0.977	0.999	0.028	0	0	0.371	Accepted	N
39	naringenin	0.018	0.972	0.997	0.576	0	0	0.742	Accepted	Y
40	naringenin 7-methyl ether ^A	0.005	0.003	0.857	0.685	0	0	0.887	Accepted	Y
41	pelargonidin	0.014	0.991	0.996	0.13	0	0	0.51	Accepted	Y
42	peonidin	0.012	0.951	0.984	0.073	0	0	0.542	Accepted	Y
43	petunidin	0.03	0.981	0.994	0.036	0	0	0.364	Accepted	Y
44	phaseollinisoflavan ^A	0.01	0.005	0.004	0.861	0	0	0.832	Accepted	Y
45	phaseolin ^A	0.015	0.002	0.003	0.719	0	0	0.79	Accepted	Y
46	p-hydroxyphenyl acetic acid	0.013	0.024	0.007	0.382	0	0	0.665	Accepted	Y
47	pinocembrin ^A	0.006	0.035	0.992	0.654	0	0	0.823	Accepted	Y
48	protocatechuic acid	0.032	0.367	0.97	0.046	0	0	0.522	Accepted	N
49	Quercetin	0.014	0.93	0.997	0.05	0	0	0.434	Accepted	N
50	Resveratrol	0.012	0.264	0.055	0.287	0	0	0.692	Accepted	Y
51	Rhamnetin	0.02	0.011	0.98	0.058	0	0	0.535	Accepted	N
52	salicylic acid	0.017	0.008	0.838	0.046	0	0	0.61	Accepted	N
53	sandosaponin aglycone	0.03	0.125	0.846	0.08	0	0	0.454	Accepted	Y
54	soyasapogenol A ^A	0.006	0.893	0.726	0.121	0	0	0.399	Accepted	Y
55	soyasapogenol B ^A	0.006	0.525	0.617	0.058	0	0	0.415	Accepted	Y
56	soyasapogenol D ^A	0.009	0.9	0.947	0.014	0	0	0.429	Accepted	Y
57	soyasapogenol E ^A	0.011	0.909	0.968	0.222	0	0	0.454	Accepted	Y
58	stigmasterol ^A	0.009	0.992	0.552	0.1	0	0	0.457	Accepted	Y
59	Taxifolin	0.014	0.892	0.678	0.039	0	0	0.501	Accepted	NP
60	Tryptophol	0.013	0.716	0.987	0.151	0	0	0.691	Accepted	N
61	vanillic acid	0.013	0.01	0.655	0.062	0	0	0.693	Accepted	N

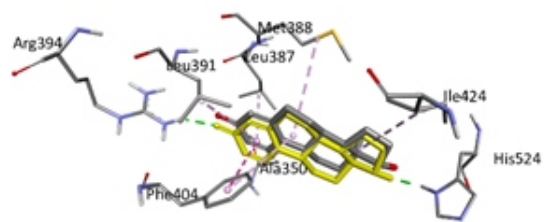
^Aisomeric SMILES strings were used in the ADMETlab2.0 web server as input

^BPresence (1) or Absence (0) of toxicophore leading to cancer via nongenotoxic mechanisms

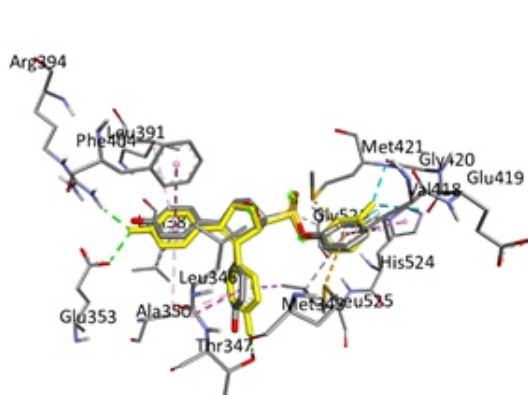
^CERA stands for ER- α and these results were obtained from Swiss Target Prediction webserver (<http://www.swisstargetprediction.ch/>); NP= no similar actives predicted in the database; Y= Yes; N= No



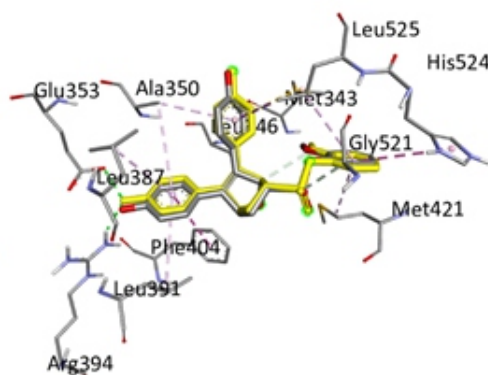
1A52



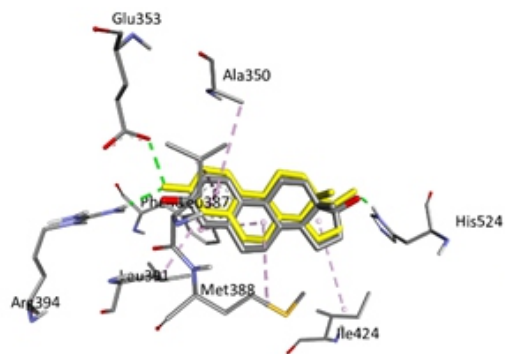
3UUD



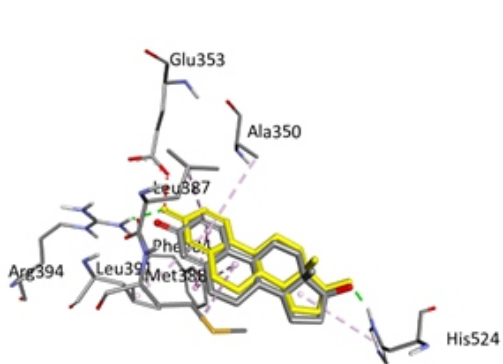
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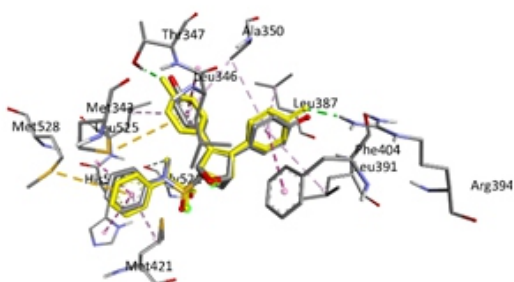
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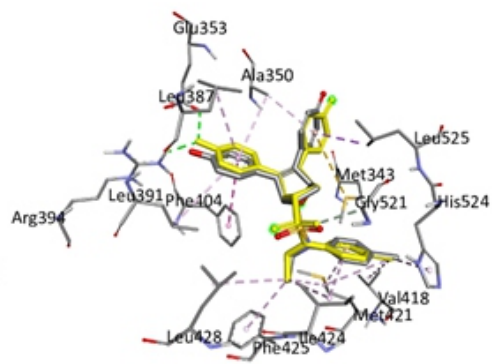
5DXE



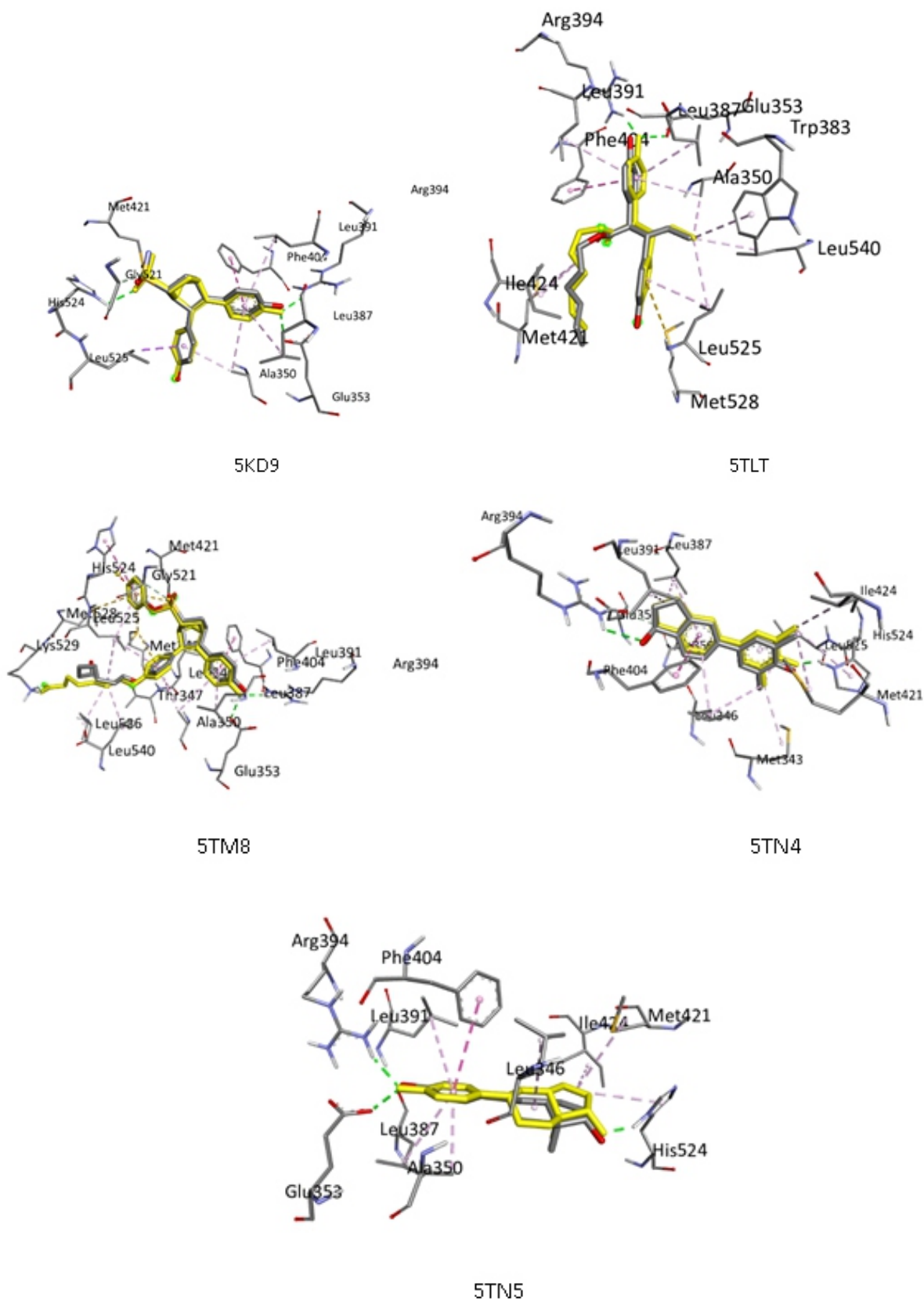
5DXG



5KCD



5KCT



Supplementary Figure 1. Superimposed images of the original and redocked poses of the co-crystallized ligands to its corresponding receptor. Shown in yellow is the original pose of the co-crystallized ligand whereas the redocked poses are shown in gray.