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An in silico study of lignans as selective estrogen receptor modulators to treat viral infections

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Abstract

Viral infections are more predominant and hazardous to human life. Treatment of viral infections poses a serious threat to the economy and the growth of society. Flax is an oil herb that constitutes lignans as the major phytocompounds. These lignans maneuver as phytoestrogens with the capability of modulating the Estrogen Receptor alpha and beta selectively. Explicitly phytoestrogens are cataloged into four main categories, such as Flavonoids, Stilbenes, Coumestans,and Lignans. Molecular docking studies were performed with 238 phytoestrogen compounds which resulted in the appreciable binding of lignans to the isoforms of estrogen receptors alpha and beta. The X ray crystal structures of human estrogen receptor α with an agonist (PDBID-1GWR), human estrogen receptor α with an antagonist (PDBID-3ERT), human estrogen receptor β with an agonist (PDBID-2JJ3) and estrogen receptor β with an antagonist (PDBID-1QKN) were used as targets. The two lignans molecules of flax seeds, Isoariciresinol and lariciresinol interacted well with both the estrogen receptors α and β. Conversely, they were analogous to an agonist with the binding score of -9.78 and -9.08 respectively with a profound binding affinity to the alternative receptors which act as agonists. They also obey the ADMET parameters with no violations of Lipinski's rules and show drug-likeliness. Isoariciresinol and lariciresinol are proficient in modulating the Estrogen Receptor Signaling. Various research papers have proved that viral replication is inhibited by the availability of estrogen to ERβ and hence can be designed as therapeutic leads for treating viral infections.

Keywords: Phytoestrogens, viral infection, SERMs, estrogen receptors, molecular docking.

Introduction

It is obvious from the past history that a deadly new disease spreads around the world that could be an erratic pandemic probably by a virus outbreak. The pandemic results in substantial morbidity, mortality and economic burdens daunting a threat to the global health concern[1]. Prevention and treatment of any novel viral illness are challenging due to the emergence of new virus strains, a high mutation rate of the existing viral strains and increasing antiviral resistance. The most common influenza virus is an RNA virus that is more challenging and re-emerging due to its RNA polymerase activity[2].

Estrogen is a female sex hormone that has anti-viral properties. It has been proved that the estrogen replacement therapy in women had been influential in fighting against the influenza virus. Estrogenic compounds have been found to reduce the replication of the virus in the nasal cavity[3]. The presence of estrogen has also inhibited the dendritic cells' maturation triggered by RNA viruses that may have reflective effects with respect to susceptibility and recovery from virus infection[4].

The two receptors of estrogen have a slightly different structure, depending on the kind of cell it is in. Estrogen receptor α (ER-α) is predominantly expressed in ovaries, endometrium, breast, liver, and heart whereas estrogen receptor β (ER-β) is found in nasal cavities, kidneys, bone, lungs and GI tract[5,6]. Selective estrogen receptor modulators (SERMs) can either be estrogen receptor agonists or antagonists depending on their activities in different tissues like bone, heart, liver, and uterine cells. Phytoestrogens are plant-derived compounds like isoflavones, stilbenes, coumestans and lignans that structurally or functionally impersonate mammalian estrogens and demonstrate possible benefits for the health of humans[7]. They can serve as impending alternative to the synthetic selective estrogen receptor modulators which are presently used in hormone replacement therapy[8]. Flaxseed (*Linum usitatissimum L.*) belonging to Linaceae family contain 35-45% linoleic and linolenic acids and few others. Flax seeds are the rich sources of lignans with the potency to act as phytoestrogens, phenolic acids, flavonoids, phenylpropanoids and tannins[9]. These seeds contain cyanogenic glycosides that protects bone, cardiac system, rich in anti cancer, antiviral, bactericidal activity, and anti-inflammatory effects, manages diabetes[10,11].

In the present study phytoestrogens of flax seeds are screened for the binding affinity to estrogen receptors alpha and beta through an *in silico* approach to find out the best lead that can

be a novel antagonist for estrogen receptor α but an agonist of estrogen receptor β. An agonist of ER- β will be beneficial in inhibiting viral activity in the nasal cells which is the predominant site of influenza infections.

Materials and methods

Target structure retrieval and preparation: RCSB Protein Data Bank is a public repository for 3 Dimensional protein structures determined either from X-ray crystallography and NMR spectroscopy. The target structures of Human estrogen receptor-α in complex with an agonist-17β Estradiol (PDBID-1GWR), human estrogen receptor in complex with an antagonist- 4-hydroxytamoxifene (PDBID-3ERT), human estrogen receptor βin complex with an agonist benzopyran (PDBID-2JJ3) and estrogen receptor β in complex with an antagonist raloxifene (PDBID-1QKN) were retrieved. The preferred proteins were chosen as they had (1) good resolution, (2) nil mutations and (3) a known ligand. The retrieved structures were then optimised with Protein preparation wizard. The receptor molecules were preprocessed by assigning bond orders, deleting water molecules beyond 5.0 A^0 and adding non-polar hydrogen atoms. The active binding sites of all the four targets were predicted, optimized and energy was minimized with OPLS force field. The optimized protein structures were validated with Ramachandran plot.

Ligand structures retrieval and optimization: A literature survey was carried out to identify the most potential phytoestrogen structures belonging to the groups' flavonoids, terpenoids, stilbenes, coumestans, and lignans were retrieved.2D structures of 238 phytoestrogens were retrieved from PubChem, a public repository to obtain small molecule structures and activity. All the molecules were optimized using ligprep wizard (Schrodinger Inc. U.S.A) and energy minimized with the OPLS3 force field.

Molecular docking: Grid-based Ligand Docking with Energetic (Glide-Schrodinger Inc. U.S.A.) version 1.8 is the software which has more accuracy in analysisng the ligand and protein and that was used for docking the ligand docking into the binding pockets of receptors. The processed receptor structure was loaded in the maestro window and receptor grid for the binding site was generated. The optimized phytocompounds were docked using the Standard Precision method onto the active sites of the four receptor structures. The interaction of the ligand and the active sites of proteins were analyzed and the best-docked ligand was identified based on the Glide energy and hydrogen bond interactions.

ADMET predictions and Quantitative Structure-Activity Relationship: Qikprop is a prediction program available in Schrodinger that quickly and accurately predicts absorption, distribution, metabolism, excretion and toxicity (ADMET) and envisages physically important descriptors and pharmaceutically applicable properties of drug molecules. ADMET predictions were performed for the prepared phytochemicals. A scatter plot was made with multiple linear regression method for the predicted and experimental properties of the ligands.

Results and discussion

The processed ligands were docked with the active site using the 'Standard precision' Glide algorithm. Out of 238 phytoestrogens from different classes, lignans topped in first ten places with their binding energy, glide score and interaction with the receptors. Glide Score is based on the Chemical Score, that includes a steric clash term and append buried polar atoms devised by Schrodinger to castigate electrostatic mismatches: Gscore=0.065*vdW + 0.130*Coul + Lipo + Hbond + BuryP + RotB + Site[12].

Validation of prepared receptors: Optimization of the four receptors resulted in the least energy of the structures and also the amino acid residues of the each of the receptors were found in the favorable regions. The Ramachandran plots for optimized receptor structures are shown in figures from 1 to 4. Fig.1, 2, 3 and 4 represent Ramachandran Plot of 1GWR, 3ERT, 2JJ3, and 1QKN respectively. The minimized energies of each of the receptors are shown in Table-1.

Table-1: Energy of the target receptors after optimization.

Target	PDB ID	Energy – OPLS3
ER alpha in complex with agonist-17 β estradiol	1GWR	-1095.255
ER alpha in complex with antagonist-4 hydroxytamoxifene	3ERT	-1088.372
ER beta in complex with agonist-benzopyran	2JJ3	-974.976
ER beta in complex with antagonist-Raloxifene	1QKN	-975.287

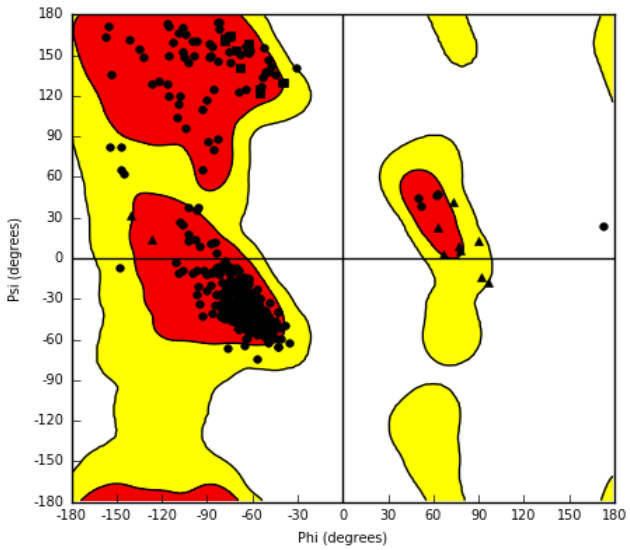

Figure-1: Ramachandran Plot of 1GWR.

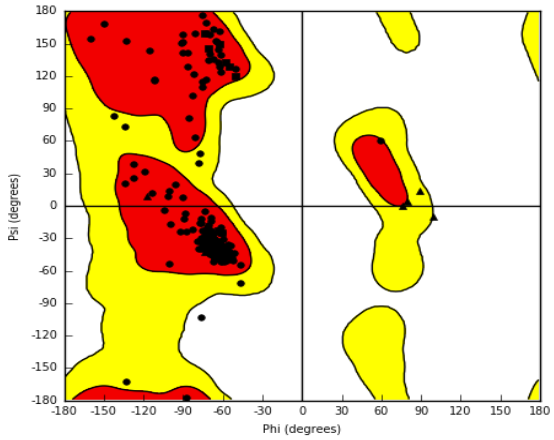

Figure-2: Ramachandran Plot of 3ERT.

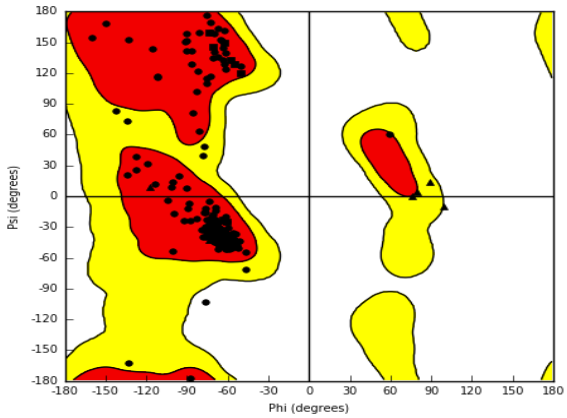

Figure-3: Ramachandran Plot of 2JJ3.

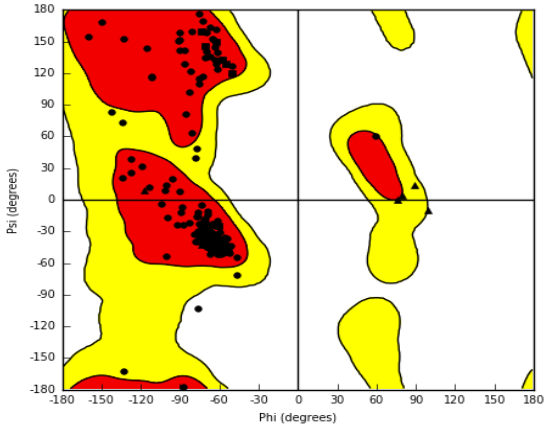

Figure-4: Ramachandran Plot of 1QKN.

Analysis of Docking: The standard precision docking algorithm resulted in the best docked ligands and poses with the Glide energy and Glide Score. Though there were many compounds that docked well with lower energy and high glide score, the best docked compounds were selected on comparing with the co-crystallized ligand that is bound to the target receptor. Among the 238 compounds, Lignans listed in Table-2, surpassed with good score, energy and also interaction with the active site residues.

Binding of isolariciresinol with Estrogen receptor α in complex with an agonist – 1GWR: Isolariciresinol of lignans was found to bind to the estrogen receptor α in the position of an agonist with the Glide energy of -73.095 and the Glide score of -9.78 and had good interaction with the active site residues of Met 421 and Met 343 with a H bond distance of 3.19A° and 3.18A° respectively. Though coumestral showed the least energy and high score, isolariciresinol can act as an agonist for ER-α –because of its interacting residues and good H bond distance. Interaction with isolariciresinol with ER-α 1GWR is shown in Figure-5.

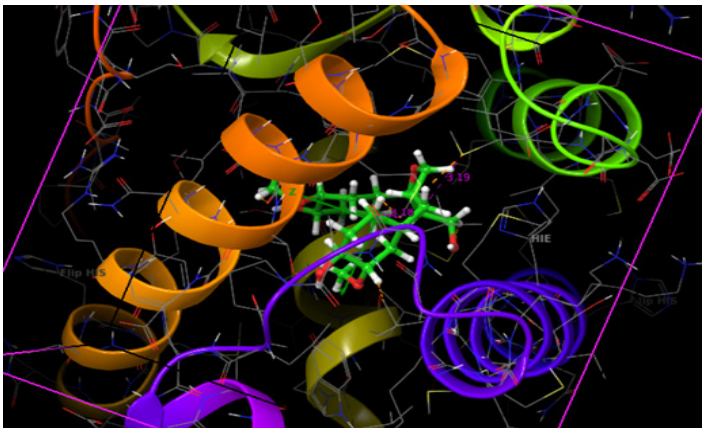

Figure-5: Docked pose of Isolariciresinol with ER alpha (agonist) 1GWR.

Binding of lariciresinol with Estrogen receptor α in complex with an antagonist – 3ERT: Lariciresinol of lignans was found to dock to the estrogen receptor α in the position of an antagonist with the Glide energy of -56.34 and the Glide score of -8.12 and had good interaction with the active site residues of Leu 387, Trp 347 and Met 343 with a H bond distance of 3.22A°, 3.14 A° and 3.26A° respectively. Thus laiciresinol is capable of potentially acting as an antagonist of ER-α. Interaction with lariciresinol with ER-α-3ERT is shown in Figure-6.

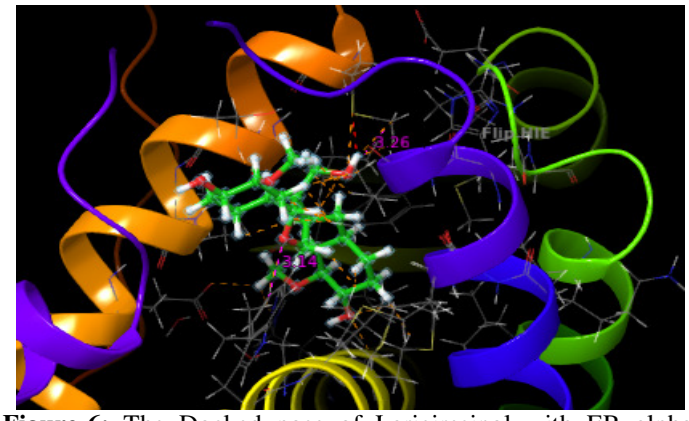

Figure-6: The Docked pose of Lariciresinol with ER alpha (antagonist) 3ERT.

Table-2: Glide Score and Glide energy of first five docked ligands.

PDBID	Compounds	Category	Glide energy	Glide Score
ER-α with agonist 1GWR	Original ligand - 17β Estradiol	Estrogen	-70.465	-9.92
	Metaresinol	Lignan	-74.085	-9.84
	Isolariciresinol	Lignan	-73.095	-9.78
	Hispidol	Aurones	-61.30	-8.94
	Secoisolariciresinol	Lignan	-35.144	-8.19
	Diasobulbin	Triterpenoids	-21.32	-8.07
ER-α with antagonoist 3ERT	4-hydroxy tamoxifene	SERM	-46.132	-8.12
	Lariciresinol	Lignan	-56.341	-7.9
	Auerisidin	Aurones	-50.129	-6.11
	Isoflavo chalcone	Chalcone	-36.281	-6.08
	Metaresinol	Lignan	-25.575	-6.5
	Isolariciresinol	Lignan	-54.38	-5.71
ER-β with agonist 2JJ3	Benzopyran	Coumarins	-65.12	-9.06
	Coumestrol	Coumestan	-72.48	-10.04
	Lariciresinol	Lignan	-64.34	-9.08
	Piceatannol	Stilbene	-53.67	-9.09
	Genistein	Isoflavone	-43.14	-8.65
	Metaresinol	Lignan	-35.14	-6.92
ER-β with antagonist 1QKN	Raloxifene	SERM	-71.73	-9.456
	Isolariciresinol	Lignan	-56.19	-8.755
	Enterolactone	Estrogen	-50.192	-8.495
	seoisolariciresinol	Lignan	-46.789	-5.308
	Coumestrol	Coumestan	-43.331	-7.308
	Biochanin A	Isoflavone	-48.35	-6.488

Binding of lariciresinol with Estrogen receptor β in complex with an agonist -2JJ3: Lariciresinol of lignans was found to dock to the estrogen receptor β in the position of an agonist with the Glide energy of -64.34 and the Glide score of -9.08 and had good interaction with the active site residues of Leu 298, Phe 309 and Phe 356 with a H bond distance of 2.90A°, 3.04 A° and 3.41A° respectively. Although coumestral has a high score and less energy, laiciresinol has the same binding affinity as the original ligand to the receptor. Hence lariciresionl is capable of potentially acting as an agonist of ER-β and its interaction with 2JJ3 is shown in Figure-7.

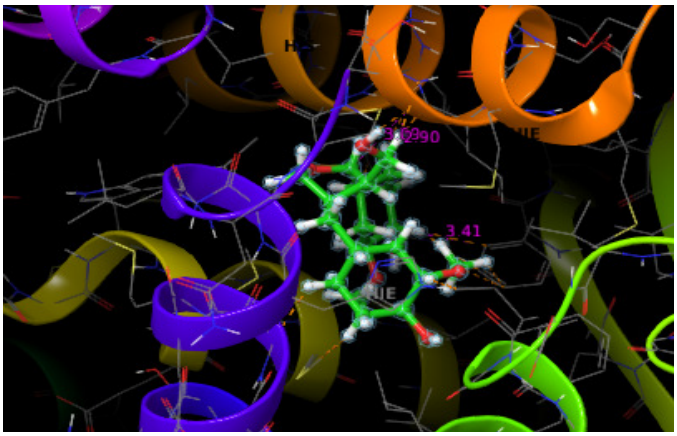

Figure-7: Docked pose of Lariciresinol with ER beta (agonist) 2JJ3.

Binding of isolariciresinol with Estrogen receptor β in complex with an antagonist- 1QKN: Isolariciresinol of lignans was found to dock to the ER- β in the position of an antagonist with the Glide energy of -56.19 and the Glide score of -8.75 and had good interaction with the active site residues of Asp258 and Leu 353 with a H bond distance of 3.13A° and 2.41A° respectively. Interaction with isolariciresinol with ER-β- 1QKN is shown in Figure-8.

Quantitative Structure-Activity Relationship of lignans present in flax seeds: Molecular properties of all the phytoestrogens were predicted using Qikprop tool. Lignans of flax seeds showed no violation of Lipinski rules of 5. Both isoariciresinol and lariciresinol demonstrated the drugable properties with percent human oral absorption of 76.58 and 86.51 respectively. Molecular weights of both these lignans are less than 500Da which is a good criterion of being a drug.

A scatter plot was drawn with the experimental logP values on X-axis and predicted logP values on Y-axis with a multiple linear regression method. Absorption Distribution Metabolism Excretion and Toxicity (ADMET) parameters (Table-3) of lignans showed that they are potential lead candidates to be able to perform as a drug. A scatter plot which represents the quantitative structure activity relationship of all the phytoestrogens that obey Lipinski's Rule of five is shown in Figure-9.

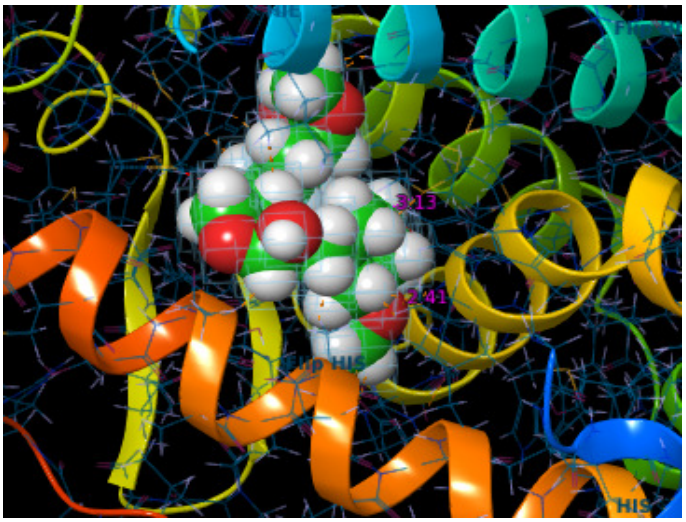

Figure-8: Docked pose of Isolariciresinol with ER beta (antagonist) 1QKN.

Table-3: ADMET properties of lignans.

Compound	Molecular weight	Donor HB	Acceptor HB	Qlog P	% human Oral absorption	Rule of 5 Violation
Metairesinol	372.501	3	10.2	-2.95	86.88	0
lariciresinol	372.500	3	10.2	-3.17	86.51	0
Pinoresinol	370.480	2	10.2	-2.84	93.38	0
Isolariciresinol	360.406	4	6.4	-3.73	78.58	0
Secoisolariciresinol	362.422	4	6.4	-2.948	82.06	0
Secoisolariciresinol Digucoside	698.801	10	27.2	-6.565	0	0
Seco Rhamnoside	520.655	6	17	-4.138	35.048	2
Iso Lariciresinol Glucoside	534.613	3	18.7	-4.643	16.80	3
Pinoresinol Digluocside	694.765	8	27.2	-6.096	0	3

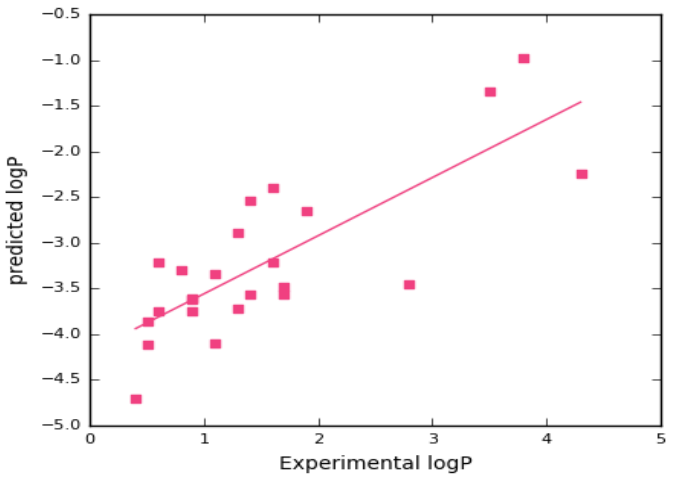

Figure-9: A Scatter plot to represent the quantitative structure activity relationship of phytoestrogens.

From the results of molecular docking, it is obvious that isolariciresinol and lariciresinol exhibited well established hydrogen bonds with the active site of the receptors. Isolariciresinol had interacted with Estrogen Receptor α as an agonist and with Estrogen Receptor β as an antagonist. Larisiresinol establishes to be an agonist for Estrogen Receptor β and an antagonist for Estrogen Receptor α which in turn avoids the formation of cancerous cells.. This agonist lariciresinol of Estrogen Receptor β helps in providing estrogen and thereby preventing viral replication. Quantitative Structure Activity Relationship suggested that these two compounds obey Lipinski's rule which states that molecular weight should be less than 500Da, partition Coeffeicient-LogP should be less than 5.0, Hydrogen bond acceptor and Hydrogen bond donor should be less than 5.0 and 10.0 respectively. Compounds Isolariciresinol and lariciresinol show promising drug-likeliness effects and are capable of functioning as selective estrogen receptor modulators.

Conclusion

Various researches have suggested that synthetic SERMs inhibited the viral replication and reduce infection. Natural SERMs, unlike synthetic ones, have variable clinical profiles, less toxicity, and little side effects. SERMs, depending on their binding properties to ERα and ERβ receptors in diverse target organs alleviate the adverse effects of Hormone replacement therapy which is currently being administered to women with estrogen deficiencies. Unlike other anti-viral agents, the lignans have anti-viral, anti-microbial properties and antioxidants that protect heart and prevent diabetes. The present study has revealed that among all the phytoestrogens studied, Lariciresinol and isolariciresinol of lignans are capable of acting as activator and inhibitor to estrogen receptors. Lariciresinol, a phytoestrogen activates ER-β thereby inhibiting the viral replication on the nasal cavity. The same phytoestrogen also acts an antagonist or inhibitor to ER-α, thereby prevents accumulation of estrogen compounds on other organs and reduces the risk of serious cancers. Protein-ligand interaction pattern play a noteworthy role in their pharmacological effect which, in turn, helps in protecting against viral infections without causing cancer. This finding can also be a leap through in novel drug development particularly in case of viral infections that are predominantly transmitted through nasal passage. The work is momentous in establishing the mechanistic overview of anti viral potentials of phytoestrogens especially lignans and predicting their activity level and need further *in vitro* and *in vivo* studies to prove the lignan bindings with estrogen receptors.

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