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Original Article

MOLECULAR MODELING AND QSAR ANALYSIS TO EXPLORE THERAPEUTIC POTENTIALS OF PHYTOESTROGENS IN OSTEOPOROSIS

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ABSTRACT

Objective: Osteoporosis is one of the most common clinical disorders, prevalent in the present scenario. Estrogen deficiency occurring after menopause or ovariectomy leads to disrupted sequence of bone remodeling. The osteoclast fusion and activation result into decrease in bone mineral density, deterioration of bone microarchitecture and increased risk of fractures.

Methods: In the present investigation, phytoestrogens were screened for their affinity towards estrogen receptor subtypes, alpha and beta through an *in silico* approach using Autodock4.2 (version 1.5.6). Further, their biological activity against ER alpha and ER beta was also predicted following Multiple Linear Regression using EasyQSAR.

Results: Spinasterol (CID_5281331), 8-prenylnaringenin (CID_480764) and Isoxanthohumol (CID_513197) were found to possess good affinity with both the estrogen receptors. It was observed that these phytoestrogen shared binding site with estradiol for ER alpha and ER beta. The predicted biological activity from QSAR analysis demonstrated that they possessed a very low EC_{50} for ER alpha and ER beta.

Conclusion: The binding pattern analysis of phytoestrogens may provide clues for design of novel agonist of the estrogen receptors with better specificity and affinity for treatment of bone related disorders.

Keywords: Bone resorption, Phytoestrogens, Osteoblasts, Osteoclasts, Molecular Docking and ADME & Toxicity.

INTRODUCTION

The bone forms an essential component of the human skeletal system. Bone tissue is considered to be dynamic as it undergoes continuous remodeling throughout lifetime. It involves replacement of fatigue bone tissue (bone resorption) by newly synthesized bone (bone formation) [1].

Bone remodeling is a highly complex and regulated phenomenon that maintains the structural integrity of the skeletal system. However during older ages, the normal sequence of the process gets disrupted leading to excessive bone resoption [1-3]. Excessive bone resorption is characterized by decrease in bone mineral density, deterioration of bone microarchitecture and increased risk of fractures [3-4].

Such a state is clinically termed as Osteoporosis. Osteoporosis occurs in both the sexes, but affects females more than the males, especially after their menopausal stage. After menopause, the decreased production of endogenous estrogen results into high bone resorption, leading to increased risk of osteoporosis [3]. Estrogen regulate the activities, metabolism and survival of bone cells including osteocytes, osteoblasts and osteoclasts through various mechanisms [3, 5].

Traditionally, women were advised to increase the consumption of the phytoestrogen-rich diet after their menopausal phase. The phytoestrogens are some of the plant derived metabolites that possess weaker, but similar estrogenic potentials, and thus forms a part of Selective Hormone Replacement Therapy (SHRT) for the treatment of osteoporosis and other bone related disorders [**3, 6-7**].

Thus, it is important to determine efficacy of various phytoestrogens for their potentials to minimize the postmenopausal bone loss in women. In this regard, we quantified the therapeutic potentials of phytoestrogens in bone related disorders through in silico approach using AutoDock4.2 by analyzing their interaction patterns with estrogen receptor subtypes.

MATERIALS AND METHODS

Protein structure retrieval & active site predictions

The X-ray crystallographic structure of the estrogen receptors was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The protein structures were cleaned using Argus Lab by removing miscellaneous ligands and other hetero-atoms such as water, ions, etc. The protein structures were further subjected to active site prediction using CASTp Calculations (Computed Atlas of Surface Topography of proteins). Amongst the predicted sites, the site containing catalytic amino acid residues (analyzed through UniProt) was selected for docking analysis.

Ligand structure retrieval

Energy minimized and optimized three dimensional structure of phytoestrogens were deduced from PubChem (http://pubchem.ncbi.nlm.nih.gov/) using PRODRG Server [**8**] (davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg).

Protein Ligand Docking

The phytoestrogens were docked onto the active site of all the selected protein models following Lamarckian Genetic Algorithm [**9**] using AutoDock4.2 software as described by Khursheed *et al* [**10**]. Further the interaction of ligand with the proteins was evaluated using LigPlot+ [**11**].

Activity Predictions

Further, the phytoestrogens were subjected to QSAR analysis applying Multiple Linear Regression methodology using EasyQSAR software. For this molecular descriptors and respective experimental EC50 values of training dataset were retrieved from e-Dragon software tool (http://www.vcclab.org/lab/edragon/start.html) and Santa Cruz Biotechnology Product Block (http://www.scbt.com/) respectively. The training dataset for ER alpha and ER beta included 9 & 8 ligand descriptors which were used to predict QSAR model. Thereafter, biological activity of 23 test dataset including phytoestrogens was predicted against Estrogen receptors based on predicted QSAR model.

RESULTS & DISCUSSIONS

Sex hormone, especially estrogen has a great impact on the overall physiology of the bone and plays a crucial role in preventing osteoporosis in women [**12-13**]. Osteoporosis is a result of imbalance in the normal bone remodeling cycle, wherein bone resorption is higher in comparison to the bone formation [**13**]. The bone formation and resorption are the characteristic activity of osteoblasts and osteoclasts respectively. The estrogen enforces a direct regulation on osteoblast and osteoclast through interaction with estrogen receptors (ER) subtypes, alpha and beta [3]. ER is nuclear hormone receptors that can initiate or enhance the transcription of genes containing hormone specific response elements. Both the subtypes of ER have been detected in osteoblasts and osteoclasts. However, Bord *et al* reported that the ER subtypes are differentially expressed in the bone [**12**]. It was generally observed that ER alpha was predominantly expressed in cortical bone, while ER beta had a higher expression in cancellous bone, thus providing a clue of their differential functionality [**12**]. Expression of ER alpha had been reported to mediate protective effect on cancellous bone by suppressing the osteoclast activity. Estrogen may also regulate the activity of osteoclast indirectly surpassing/enhancing the release of osteoclast stimulatory/inhibitory factors respectively (Figure 1). Estrogen inhibits bone resorption by down regulating the expression of RANK ligand (RANKL) and upregulating the Osteoprotegrin (OPG) in osteoblasts and bone marrow stromal cells. OPG binds to RANKL, making it unavailable to interact with osteoclast receptor, RANK [**5, 14**]. It is also important to mention that estrogen also blocks the expression of MCSF gene by regulating phosphorylation of Egr-1 and its interaction with Sp-1 [**15**]. Both these processes prevent fusion and differentiation of osteoclast precursors. Recent reports also suggest that estrogen may promote the apoptosis of the osteoclasts like cells via TGF beta mediated pathway [**14, 16**]. Apart from this, estrogen also decreases the expression of IL-1, IL-6 and TNF beta from bone marrow stromal cells and osteoblast, which play a crucial role in osteoclast stimulation and bone resorption [**5, 13, 16**]. Kameda *et al* also reported a reduced expression of Cathepsin K (CatK), a serine protease responsible for degradation of collagen type I with the estrogen treatment [**16**].

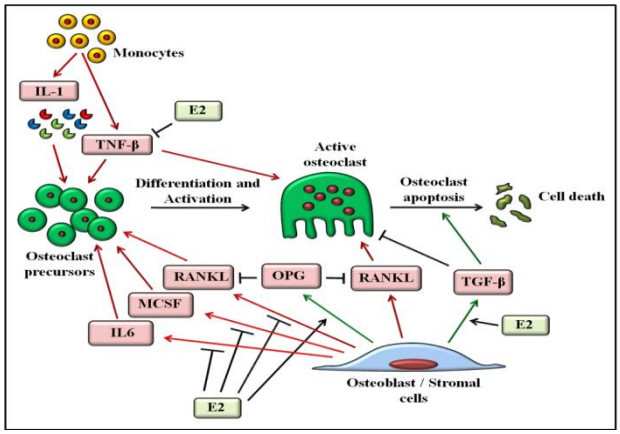

Fig. 1: Mechanistic overview: regulatory role of estrogen (E2) in bone homeostasis. Red arrows represents activities that promote to bone resorption, while green represents those that inhibit bone resorption. (For details refer text)

The pathological bone loss caused by estrogen deficiency after menopause or ovariectomy can be prevented by selective estrogen replacement therapy (SERT). This therapy includes the application of such chemical modulators that possess similar estrogenic properties so as to compensate the estrogen deficiency, thus preventing the bone loss [**17**]. Amongst many synthetic modulators available, phytoestrogens are natural plant derived metabolites, known for their weaker yet similar estrogenic activity [6]. Beck *et al* reported that phytoestrogens also exert their estrogenic activity through their interaction with (ER's) [6]. Such interaction of the phytoestrogens may be due to their structural similarity with the estradiol (17- beta estradiol), a natural ligand of estrogen receptor. Structural analysis of the phytoestrogens revealed the presence of key structural elements such presence of phenolic ring and position of hydroxyl groups which were found to be similar to estradiol (Figure 2).

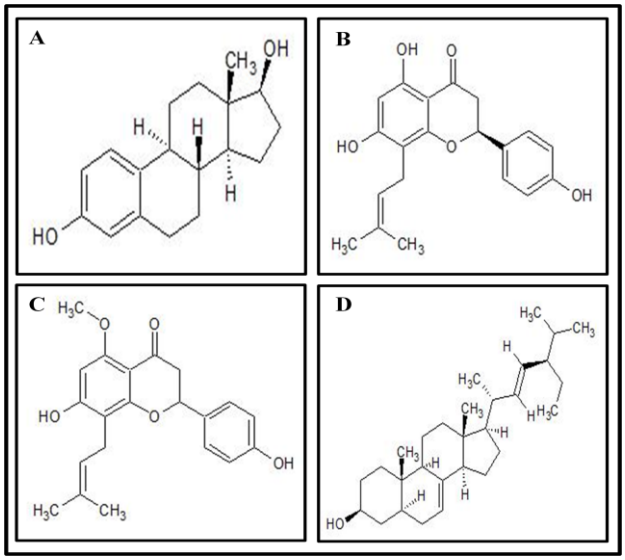

Fig. 2: Chemical structures of phytoestrogens. A) 17-β-estradiol (CID_5757), B) 8-prenylnaringenin (CID_480764), C) Isoxanthohumol (CID_513197), D) Spinasterol (CID_5281331).

In the present investigation, in order to screen out the therapeutic potentials in bone resorption related disorders, phytoestrogens were evaluated using *in silico* approach through AutoDock4.2 software. The structural and functional relationship of phytoestrogens was evaluated against the three dimensional structures of estrogen receptors (alpha and beta) retrieved, from Protein Data Bank. In order to modulate the activity of protein, a ligand must interact with either protein's active site or the allosteric site residues present in the protein. In this regard, the active site of the protein forms a prime target for novel drug design. Analysis of the amino acid residues and their dynamics allow mapping and improving of the interaction profile of ligands with the protein.

The 3D structures of phytoestrogens isolated from various plant sources were screened for their osteogenic potentials. Interaction analysis of phytoestrogens demonstrated that Spinasterol (CID_5281331) had highest binding affinity with ER alpha (estimated binding energy (BE): -9.22kcal/mol) while 8-prenylnaringenin (CID_480764) possessed highest binding affinity with ER beta (BE: -10.48kcal/mol). 8-prenylnaringenin was also found to possess high affinity for ER beta (BE: -6.9kcal/mol) and ER alpha (BE: -8.79kcal/mol) respectively. In comparision, estradiol (17-beta-estradiol) was found to possess a lower affinity with ER alpha BE: -7.69kcal/mol) and ER beta (BE: -9.78kcal/mol). The interaction of estradiol was found with Glu323, Glu353 & Trp393 of ER alpha and Leu339, Gly472 & His475 of ER beta. The binding pattern of spinasterol and 8-prenylnaringenin with estrogen receptors differ completely. However, spinasterol shared binding site at Glu353 with estradiol in case of ER alpha, while 8-prenylnaringenin shared site at Gly472 with ER beta. It also important to mention that Isoxanthohumol (CID_513197) also possess a good affinity with both the estrogen receptors ((BEERalpha: -8.92kcal/mol) & (BEERbeta: -9.15kcal/mol)). It was observed that Isoxanthohumol shared binding site at Trp393 with ER alpha and at His475 with ER beta (Figure 3). volumes (Sv), sum of atomic Sanderson electronegativities (Se), sum of atomic polarizabilities (Sp), sum of Kier-Hall electrotopological states (Ss),

mean electrotopological state (Ms), Moriguchi octanol-water partition coefficient (MLOGP) and Ghose-Crippen octanol-water partition coefficient (ALOGP) with Rsq = 0.9803, adjusted Rsq = 0.8425, F statistics = 7.11 and critical F = 3.50. All the descriptors except Ms demonstrated a negative correlation with the activity. It is also important to mention ALOGP contributed in activity to higher extent with percentage contribution greater than 50%. Similarly for ER beta sum of atomic vander Waals volumes (Sv), mean electrotopological state (Ms), mean atomic Sanderson electronegativity (Me), mean atomic vander Waals volume (Mv), Moriguchi octanol-water partition coefficient (MLOGP) and Ghose-Crippen octanol-water partition coefficient (ALOGP) were taken as descriptors for model prediction with Rsq = 0.9934, adjusted Rsq = 0.9539, F statistics = 25.12 and critical F = 3.87.

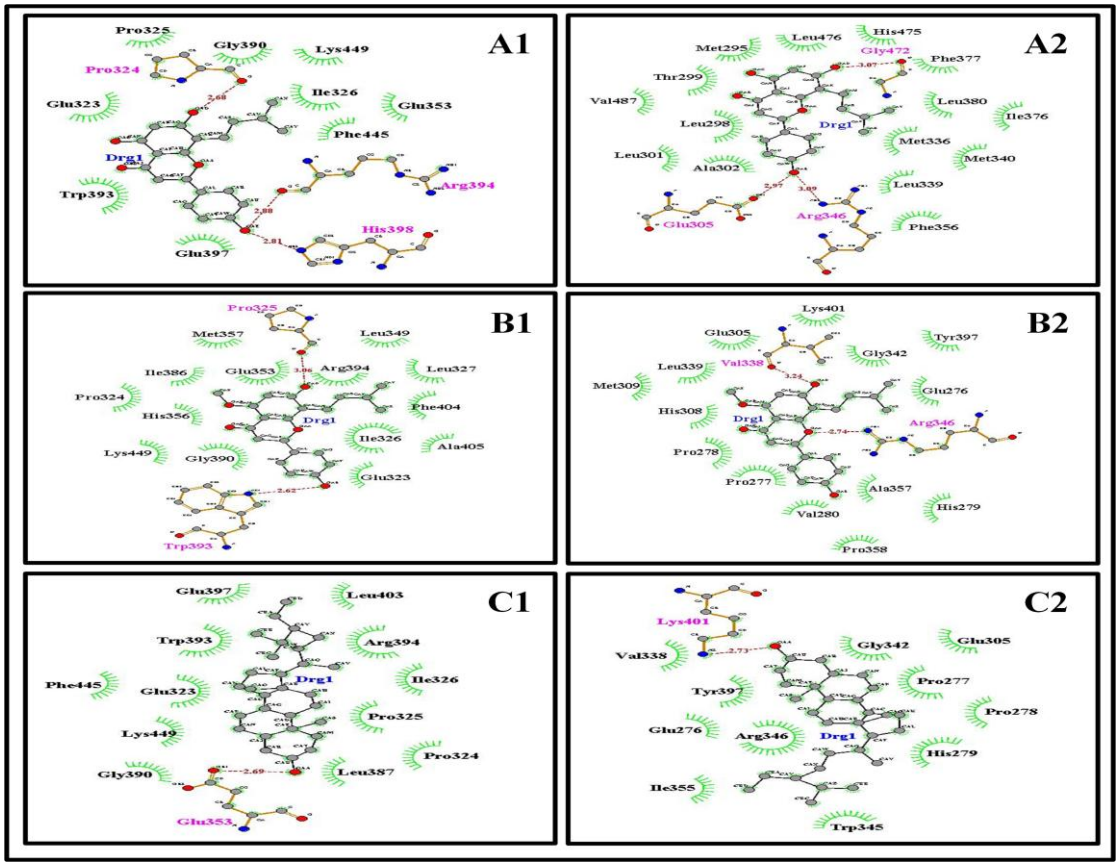

Fig. 3: Molecular interaction pattern of phytoestrogens with the ER alpha (1) and ER beta (2): A) 8-prenylnaringenin (CID_480764), B) Isoxanthohumol (CID_513197), C) Spinasterol (CID_5281331).

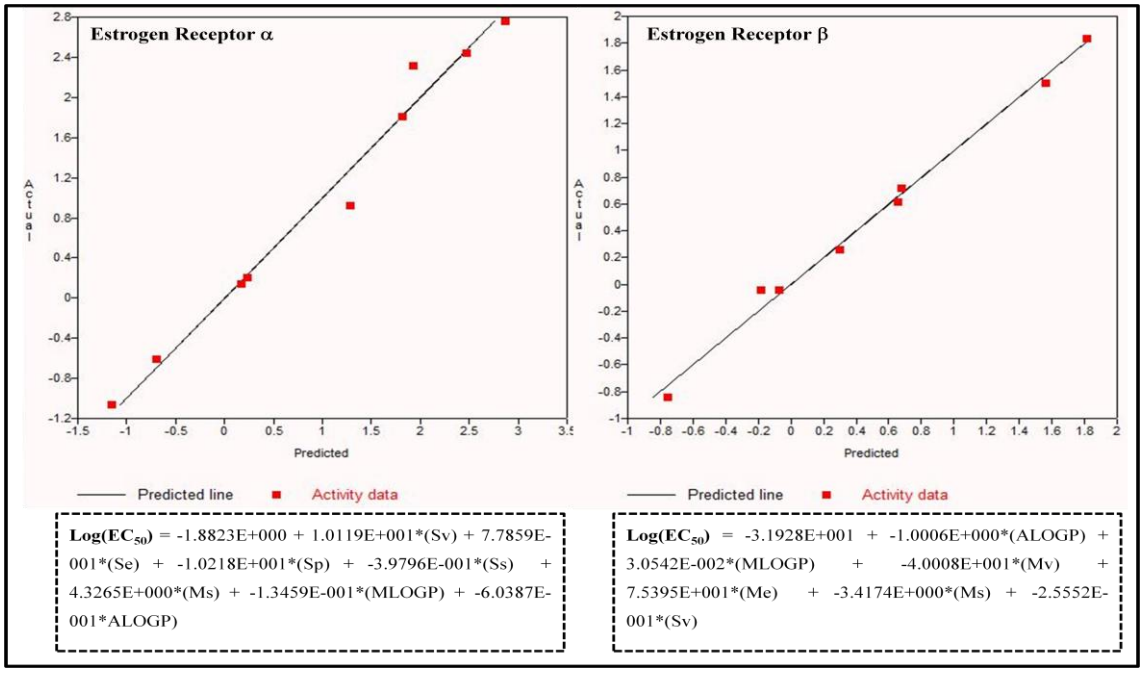

Fig. 4: QSAR Activity plot and governing equation for ER alpha and ER beta.

Table 1: Predicted EC50 and corresponding binding affinity of phytoestrogens with ER alpha

S. No.	KNApSAcK-3D/ PubChem ID	ER		
		log EC_{50}	predicted EC_{50} (nM)	Binding Energy (kcal/mol)
1	65373	0.49	3.09	-6.13
2	89472	1.64	43.65	-6.63
3	91469	2.32	208.93	-6.7
4	115089	0.57	3.71	-6.66
5	119205	0.96	9.12	-7.6
6	155094	-0.33	0.47	-7.83
7	250817	2.16	144.54	-6.63
8	261166	-0.04	0.91	-5.66
9	480764	-0.33	0.47	-8.79
10	513197	-0.36	0.44	-8.92
11	639665	-1.53	0.029	-6.35
12	5280373	2.99	977.24	-7.73
13	5280378	2.73	537.032	-7.37
14	5280961	3.21	1621.81	-7.42
15	5281331	-5.78	1.65959E-06	-9.22
16	5281576	0.32	2.089	-7.21
17	5281707	3.38	2398.83	-6.99
18	5281708	3.06	1148.16	-6.99
19	5317750	3.06	1148.16	-7.83
20	5319565	3.2	1584.89	-6.88
21	5487671	3.37	2344.29	-7.13
22	10685477	0.9	7.94	-7.43
23	5757	0.14	1.38	-8.62

Table 2: Predicted EC50 and corresponding binding affinity of phytoestrogens with ER beta

S. No.	KNApSAcK-3D/ PubChem ID	ER		
		log EC_{50}	predicted EC_{50} (nM)	Binding Energy (kcal/mol)
1	65373	1.35	22.38	-5.18
2	89472	0.94	8.71	-8.17
3	91469	1.83	67.61	-8.89
4	115089	0.89	7.76	-6.42
5	119205	0	1	-7.76
6	155094	-1.07	0.085	-6.95
7	250817	0.07	1.175	-7
8	261166	-1.43	0.037	-8.75
9	480764	-1.07	0.085	-10.48
10	513197	-1.36	0.044	-9.15
11	639665	-2.15	0.007	-6.14
12	5280373	2.02	104.71	-8.62
13	5280378	1.24	17.38	-8.23
14	5280961	2.48	301.99	-8.8
15	5281331	-5.79	1.62181E-06	-6.9
16	5281576	1.24	17.38	-8.01
17	5281707	0.97	9.33	-8.7
18	5281708	1.7	50.119	-8.63
19	5317750	2	100	-8.72
20	5319565	0.47	2.95	-8.67
21	5487671	1.27	18.62	-8.59
22	10685477	0.33	2.138	-9.36
23	5757	0.62	4.17	-9.47

Here also, ALOGP contributed nearly to 50% in determining activity of ligand. Validation of QSAR model was done by comparing the actual log(EC_{50}) and predicted log(EC_{50}) of the training dataset. The model QSAR equation and plot of actual and predicted activity for ER alpha and ER beta have been demonstrated in (Figure 4). Further, EC_{50} values of the test dataset were analyzed using predicted QSAR model ER alpha (Table 1) and ER beta (Table 2). Prediction of EC_{50} of the test dataset demonstrated that the phytoestrogens possessed lower EC_{50} for ER beta in comparision to ER alpha. However, 17 beta estradiol showed a slight better efficacy for ER alpha. The lower EC_{50} of 8-prenylnaringenin (CID_480764) and Isoxanthohumol (CID_513197) directly correlated with the earlier performed docking studies.

CONCLUSION

In recent years, substantial amount of researches to combat bone related diseases, had made a tremendous impact over the world. Yet, the urge for development of novel leads with such biological activity sustains in the society. In this regard, phytoestrogens (used traditionally for treatment of bone related diseases) may provide a scaffold for the discovery of new hits. Protein ligand interaction pattern play a significant role in their pharmacological effect. *In silico* approach of molecular docking provides best platform to analyze such interactions. The work is significant in establishing the mechanistic overview of osteogenic potentials of phytoestrogens and predicting their activity level. Our results demonstrated that phytoestrogens may exert their osteogenic effect by stimulating estrogen receptors. Further, *in vivo* and *in vitro* validation of the studies is required.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

"NOTE: The First Author and Second Author have contributed equally."

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