Original Article

In Silico Studies of Isoflavones as Estrogen Receptor α (ERα) Activators Targeting Cardiovascular Diseases

Raju Bairi[1], Vadivelan Ramachandran*[1], Kalirajan Rajagopal[2], Srikanth Jupudi[2]

[1] Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research (JSSAHER), Ooty-643001, Tamilnadu, India.

[2] Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research (JSSAHER), Ooty-643001, Tamilnadu, India.

E-mail: vadivelanr@jssuni.edu.in

Abstract

Introduction: Isoflavones are plant derived chemical substances and are called as phytoestrogens. These are present in different parts of the plants belongs to legumes family and chemical structures are resembled with endogenously available female reproductive hormone estradiol. Isoflavones exerts their biological actions by activates estrogen receptors as estradiol.

Methods: The in-silico docking studies were performed for isoflavone compounds as ERα (Estrogen Receptor Alpha) (PDB ID- 2IOG) activators targeting cardiovascular diseases using Schrodinger suite 2019-2, Maestro 9.6 version. Docking against ERα was performed using glide module, in silico ADMET screening by qikprop module and free binding energy by prime MMGBSA module.

Results: The binding affinity of the compounds towards ERα was selected by glide score and interaction patterns. Many compounds showed strong hydrophobic interactions and hydrogen bonding interactions to activate ERα as standard substance estradiol. The compounds sissotrin, artocarpin, warangalone, puerarin, alpinum and luteone have good binding affinity with glide scores -11.91, -11.68, -11.55, -11.43, -11.26 and -11.10 respectively when compared with standard compound i.e estradiol glide score was -9.85. The ADMET properties are with in recommended values. MMGBSA binding results of the most potent activators are favourable.

Conclusion: The compounds sissotrin, artocarpin, warangalone, puerarin, alpinum and luteone with good glide scores may produce significant cardioprotective activity like estradiol and further in in vitro and in vivo investigations.

Keywords: Docking, Estradiol, Glide score, Phytoestrogens, qikprop

DOI: 10.47750/pnr.2022.13.S04.118

INTRODUCTION

Cardiovascular diseases (CVDs) are the number one cause of death globally, taking an estimated 17.9 million lives each year. [1,2] The structural and functional changes in heart and blood vessels may leads to CVDs such as coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. Oxidative stress is the most common cause of ischaemic injury leads to CVDs due to the failure of antioxidative mechanism in a cell and apoptosis is another factor causes myocardial ischaemia and heart failure. [3,4]

According to epidemiological studies premenopausal women are less predisposed to cardiovascular risks compared to age matched men. Female protection against CVD is associated with sex hormone levels, as the incidence and severity of CVD increases in postmenopausal women. Worldwide women are suffering with reduced quality of life by menopausal symptoms. Estrogen levels are declined in postmenopausal condition leads to vasomotor symptoms combine with increased probabilities of bone fractures, cardiovascular problems and changes in blood cholesterol levels. Modern treatment for vasomotor symptoms is hormone replacement therapy (HRT) but this therapy causes risk of cancer in uterus, breast and ovaries because of the tissues were rich in ERα. [5] Estrogen and estrogen receptors are mediating this cardio protection but the complex mechanism is unclear. Activation of estrogen receptors mediates genomic and nongenomic actions induces cardioprotection in myocardial cell in ischaemic reperfusion injury. [6-9]

There are two types of estrogen receptors such as estrogen receptor α (ERα) and estrogen receptor β (ERβ) are localized in human cardiac cells of male and females. ERα and ERβ both are play an important role in CVDs modulation through the activation of kinases such as PI3K, MAPK and eNOS activation results in NO production. Excessive NO released in heart and blood vessels exerts cardiovascular protective mechanisms such as vasodilation, platelet inhibition and survival of endothelial cells. Estrogen exerts cardio protection by multiple signaling pathways through the activation of ERα and ERβ receptors. [8,10-

16]

Some pathological conditions raised in cardiovascular system like myocardial infarction, ischaemic reperfusion injury and heart failure occurs due to oxidative stress, to counteract this therapeutic approach is either administration of antioxidant substance or increase the availability of endogenous antioxidants. [4,17]

Isoflavones are one of the class of phytoestrogens and are obtained from plants belongs to legumes. Phytoestrogens produce estrogenic and antiestrogenic actions interact with estrogen receptors based on endogenous estrogen levels. [4,18,19]

Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. [20,21] Docking of isoflavones into the binding site of an estrogen receptor reveal the binding affinity of the complex which is a key part in structure-based drug designing. [22] The present investigation aims to determine the interaction pattern of isoflavones with human estrogen receptors (ERα and ERβ) using Schrodinger suite 2019-2.

Material And Methods

Protein preparation

The Estrogen Receptor α (ER α) protein with co crystallised ligand (PDB ID: 2IOG) was retrieved from the RCSB protein data bank (www.rcsb.org). The above protein was prepared by protein preparation wizard module of Schrodinger suite 2019-2. Water molecules without hydrogen bonds are deleted. Missing chain atoms are added by using prime module of Schrodinger suite 2019-2. The possible ionisation states were generated for the heteroatom present in the protein structure and the most stable state was chosen. Finally, a restrained minimisation of the protein structure was carried out using OPLS3 force field to reorient side-chain hydroxyl groups and alleviate potential steric clashes. A grid box was generated to defined the centroid of the active site for docking studies.[23]

Receptor Grid Generation

The co-crystallized ligand was held in the gem structure of the protein arranged from protein planning wizard and it was utilized for the receptor lattice development. A Gride box was produced to characterize the centroid of the dynamic site which is utilized for docking. The Grid box is produced by utilizing the Glide grid generation wizard. The Glide grid box measurements (the centroid of a docked posture) of the protein were set to 14Ao x 14Ao x 14 Ao.

Ligand preparation

The structures of the ligands were generated and subjected to LigPrep module of Schrodinger suite 2019. They were converted from 2D to 3D structures by including stereo chemical, ionisation, tautomeric variations, as well as energy minimisation and optimised for their geometry, desalted and corrected for their chiralities and missing hydrogen atoms. The ionisation and tautomeric states were generated between pH of 6.8 to 7.2 using Epik module. In the final stage of LigPrep, compounds were minimised using optimised potentials for liquid simulations 3 (OPLS3) force field in impact package of Schrodinger until a root mean square deviation of 1.8A0 was achieved. A single low energy ring conformation per ligand was generated and the optimised ligands were used for docking analysis.

Glide ligand docking

The designed compounds were docked into the catalytic pocket of ER α protein (PDB ID: 2IOG) by using Glide module of Schrodinger suite 2019-2. The best docked compounds were selected by using the Glide score function. The favourable interactions between ligand molecules and the receptor were scored using Glide ligand docking program. All the docking calculations were performed using extra precision (XP) mode and OPLS3 force field. The above docking process was run in a flexible docking mode which automatically generates conformations for each input ligand. This algorithm recognises favourable hydrophobic, hydrogen-bonding and metal-ligation interactions, and penalises steric clashes. Finally, the minimised poses were re-scored using Glide Score scoring function. The XP-Glide score of active compounds were summarised and compared with the Glide score of standard compound estradiol.

The in-silico ADME properties of the proposed compounds were determined by qikprop of Schrodinger suite 2019-2.

Binding free energy calculation by using prime/MM-GBSA approach

The binding free energies of the complex were computed by molecular mechanics generalised born surface area (MM-GBSA) using the Prime module of Schrodinger suite 2019-2 which incorporate the OPLS3 force field and VSGB solvent model to search algorithms.

Results And Discussion

The molecular docking studies of the isoflavone compounds to protein active sites were performed by using an advanced molecular docking program in Schrodinger suite 2019-2 for determining the binding affinities of the ligands. The selected isoflavones were docked in to the estrogen receptor α (PDB ID: 2IOG) in order to ascertain their potential ERα stimulation activity against cardiovascular risks. G- score was used to access the binding affinities of the studied compounds and standard compound to the target receptor and the results are depicted in Table 1. The G-scores of selected isoflavones range from -11.91 to -7.72 and standard compound estradiol G-score was -9.85. Out of 55 compounds 27 compounds showed good affinity to the receptor when compared to the standard. The G-scores of the 27 compounds range from -11.91 to -9.89 and six compounds affinity score was above 11.0 compared to standard molecule estradiol was shown in Figure 1a to 1g. The docking results reveal that the interactions are mainly due to the lipophilic factors due to the presence of phenyl ring and chromen-4-one ring.

The ligand and protein molecule interactions were depicted was shown in figure 2a to 2g. Ligand 5280781(Sissotrin) two hydroxyl groups of glycan moiety formed two hydrogen bonds with Glu353 and Leu387. The p-hydroxy group on the phenyl ring of 5458461(Artocarpin) attached to the 2nd position of chromen-4-one ring formed one hydrogen bond with Glu353, the phenyl ring formed hydrophobic interaction with Phe404. The p-hydroxy group on the phenyl ring of the compound 5379679 (Warangalone) attached to the 3rd position of chromen-4-one ring formed two hydrogen bonds with Leu387 and Arg394, the phenyl ring formed hydrophobic interaction with Phe404. The ligand 5281807 (Puerarin) showed the two hydroxyl groups of glycan moiety formed two hydrogen bonds with Glu353 and Leu387. In case of the ligand 5490139 (Alpinum) the p-hydroxy group on the phenyl ring attached to the 3rd position of chromen-4-one ring formed two hydrogen bonds with Leu387 and Arg394, the phenyl ring formed hydrophobic interaction with Phe404 and the ligand 5281797 (Luteone) p-hydroxy group on the phenyl ring attached to the 3rd position of chromen-4-one ring formed two hydrogen bonds with Glu353 and Arg394. The 7th hydroxyl group interacted with Gly521. The phenyl ring formed hydrophobic interaction with Phe404. The 3rd hydroxyl group of estradiol formed two hydrogen bonds with Arg394 and leu387. The aromatic ring formed π-π stacking interaction with Phe404.

The isoflavones are oriented so that the phenyl ring attached to the 3rd or 2nd position of chromen-4-one to form hydrogen bonds with Glu353, Leu387 and Arg394 and also the hydrophobic interaction with Phe404 also stabilizes the phenyl ring as estradiol. Whereas, isoflavones bound to glycone moiety are oriented so that glycone hydroxyl groups formed hydrogen bonds with Glu353, Leu387 and Arg394.

Physicochemical properties and pharmacokinetic (ADMET) parameters provide druglike properties of selected molecules. Pharmacokinetics and to studies of selected compounds by using computational analysis results depicted in Table 2. The predicted parameters are QP log Po/w, QP log HERG, QPP Caco, QP log BB, QPP MDCK, QP log Kp, QP log Khsa and Percent Human Oral Absorption (PHOA). All values of tested compounds are compared with known isoflavones present in the group such as daidzein, formononetin and genistein. Except iridin all molecules percentage of bioavailability with in the normal range.

Molecular docking was additionally assessed with MM-GBSA free restricting vitality which is identified with the post scoring approach for estrogen receptor α (PDB ID: 2IOG) target. The accuracy of docking is confirmed by examining the lowest energy poses predicted by the scoring function. The Glide scores almost resemble the experimental binding mode as determined by X-ray crystallography. The Glide score and MM-GBSA free energy esteems are obtained by the docking of ligands into the coupling pocket. The subtleties of the MM-GBSA free restricting vitality for the compounds are shown in Table 3.

Conclusion

Nature providing so many beneficial phytochemicals to the living organisms in the form of plants, animals and others. Traditionally these plants are widely used in different health disorders as remedies. Worldwide population consuming medicinal plants and plant materials as part of their diet living healthy. Isoflavones are a class of phytoestrogens widely present in plants belongs to the family of Leguminosae. Soy is the one of the richest sources of isoflavones includes genistein, daidzein and glycitein. Epidemiological studies showing that administration of soy isoflavones protects human health from various diseases like endogenous estrogen hormone includes menopausal symptoms, osteoporosis, cardiovascular risks diabetes and cancer.

Western population are more prone to cardiovascular diseases compred to Asian population due to less consumption of soy food. This study has revealed potent estrogen receptor activators with good receptor binding affinity and predicted pharmacokinetic profiles that may be further investigated for their in vitro as well as in vivo activity towards estrogen receptor mediated cardioprotection.

Table 1: Docking studies for selected compounds with Estrogen Receptor α (2IOG.pdb)

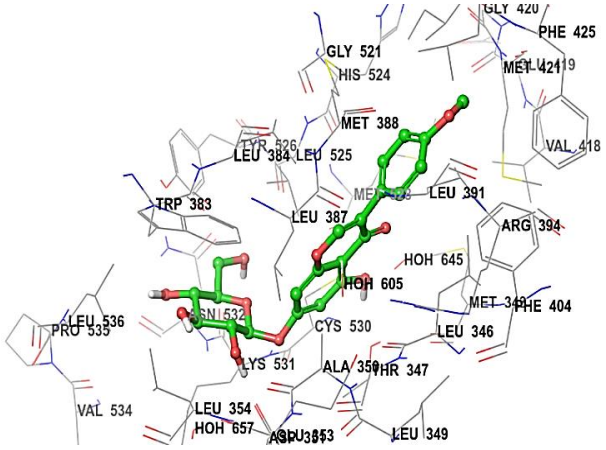
S. NO	Ligand	GScore	Lipophilic EvdW	PhobEn	HBond	Electro	LowMW	Penalties	RotPenal
	Sissotrin	-11.91	-6.45	-1.62	-3.2	-0.8	-0.01	0	0.17
	Artocarpin	-11.68	-7.72	-2.4	-1.28	-0.4	-0.05	0	0.16
	Warangalone	-11.55	-7.57	-2.18	-1.55	-0.25	-0.15	0	0.15
	Puerarin	-11.43	-6.48	-1.95	-2.48	-0.56	-0.11	0	0.15
	Alpinum	-11.26	-6.53	-2.7	-1.55	-0.17	-0.38	0	0.07
	Luteone	-11.1	-6.43	-1.94	-1.68	-0.86	-0.32	0	0.14
	Gancaonin L	-10.98	-6.02	-2.26	-1.64	-0.88	-0.32	0	0.14
	Neobavaisoflavone	-10.96	-6.23	-2.55	-1.18	-0.74	-0.43	0	0.16
	Orobol	-10.95	-4.67	-2.31	-2.38	-1.16	-0.5	0	0.07
	LachnoisoflavonB	-10.89	-6.47	-2.7	-0.98	-0.45	-0.33	0	0.05
	Glabrone	-10.79	-6.21	-2.7	-1.4	-0.17	-0.38	0	0.07
	Dalparvone	-10.79	-5.99	-2.17	-2.03	-0.28	-0.4	0	0.07
	Isowighteone	-10.65	-6.14	-1.78	-1.51	-1	-0.37	0	0.15
	Bavachinin	-10.52	-6.25	-2.56	-0.69	-0.53	-0.37	0	0.15
	Pomiferin	-10.51	-6.15	-2.08	-1.76	-0.56	-0.1	0	0.14

	Glabranin	-10.45	-6.37	-2.7	-0.71	-0.16	-0.42	0	0.23
	Koparin	-10.44	-5.51	-2.7	-1.69	-0.12	-0.5	0	0.09
	Albanin A	-10.41	-6.93	-1.54	-1.15	-0.66	-0.32	0	0.2
	Iridin	-10.26	-6.66	-0.32	-3.6	-0.56	0	1	0.13
	Irigenin	-10.23	-6.42	-2.08	-1.44	-0.05	-0.3	0	0.06
	Calycosin	-10.18	-5.48	-2.7	-1.38	-0.21	-0.5	0	0.1
	Irisflorentin	-10.16	-6.9	-2.66	0	-0.04	-0.21	0	0.06
	Morusin	-10.04	-7.14	-2.7	-1.31	-0.44	-0.1	1.5	0.14
	Genistein	-10.03	-4.85	-2.6	-1.59	-0.56	-0.5	0	0.07
	Biochanin A	-9.99	-5.65	-2.47	-1.31	-0.16	-0.5	0	0.1
	Tectorigenin	-9.96	-5.09	-2.41	-1.53	-0.5	-0.5	0	0.06
	Pinostrobin	-9.89	-5.21	-2.7	-0.96	-0.39	-0.5	0	0.1
	Pratensein	-9.83	-5.38	-1.73	-1.62	-0.7	-0.5	0	0.09
	Licoflavanone	-9.83	-6.54	-1.66	-0.83	-0.44	-0.37	0	0.21
	Formononetin	-9.78	-5.75	-2.7	-0.79	-0.15	-0.5	0	0.11
	Poriol	-9.77	-4.31	-2.31	-1.67	-0.76	-0.5	0	0.07
	Irilone	-9.75	-4.84	-2.7	-0.98	-0.38	-0.5	0	0.06
	Caragiside A	-9.75	-7.07	-0.57	-1.92	-0.08	0	0	0.16

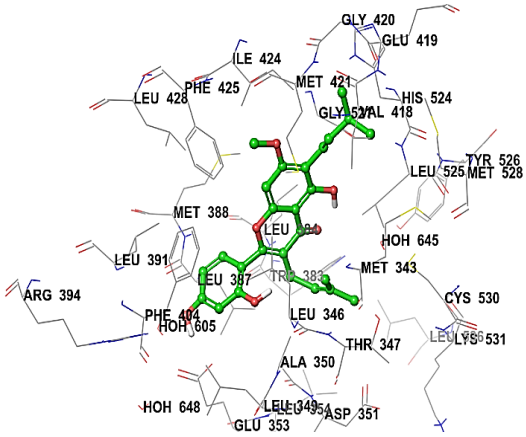
	Morin hydrate	-9.7	-4.46	-1.62	-2.64	-0.55	-0.49	0	0.06
	Glisoflavone	-9.64	-5.76	-1.48	-1.73	-0.58	-0.27	0	0.18
	Pseudobaptigenin	-9.62	-5.38	-2.7	-0.56	-0.18	-0.5	0	0.1
	Cajanin	-9.59	-5.01	-2.01	-1.62	-0.52	-0.5	0	0.06
	Daidzein	-9.58	-4.78	-2.53	-1.14	-0.7	-0.5	0	0.08
	Glycitein	-9.56	-5.01	-2.7	-0.94	-0.48	-0.5	0	0.07
	Cladrin	-9.51	-5.48	-2.7	-0.52	-0.37	-0.5	0	0.06
	Vestitone	-9.51	-5.21	-2.35	-0.79	-0.46	-0.5	0	0.07
	Datiscetin	-9.5	-4.72	-1.84	-1.76	-0.77	-0.5	0	0.09
	Prunetin	-9.48	-5.51	-2.13	-1.23	-0.21	-0.5	0	0.1
	Chrysin	-9.47	-4.77	-2.7	-1.18	-0.44	-0.5	0	0.12
	Ermanin	-9.44	-5.88	-1.86	-0.96	-0.38	-0.45	0	0.08
	Pratol	-9.43	-5.28	-2.54	-0.7	-0.52	-0.5	0	0.11
	Icaritin	-9.37	-6.52	-1.71	-0.96	-0.08	-0.27	0	0.18
	Bowdichione	-9.31	-5.27	-2.34	-0.51	-0.21	-0.5	0	0.09
	Tectoridin	-9.28	-5.95	-0.61	-3.1	-0.53	0	1	0.16
	Isosakuranetin	-9.13	-5.15	-1.89	-0.83	-0.46	-0.5	0	0.09
	Steppogenin	-8.94	-4.34	-1.91	-2.18	-0.77	-0.5	1	0.07

	Pinocembrin	-8.8	-4.74	-2.03	-0.83	-0.4	-0.5	0	0.12
	Norartocarpetin	-8.71	-4.93	-1.89	-2.33	-0.64	-0.5	1.5	0.09
	Medicarpin	-7.72	-4.21	-1.92	-0.7	-0.39	-0.5	0	0
	Std.Estradiol	-9.85	-5.89	-2.7	-0.61	-0.15	-0.5	0	0

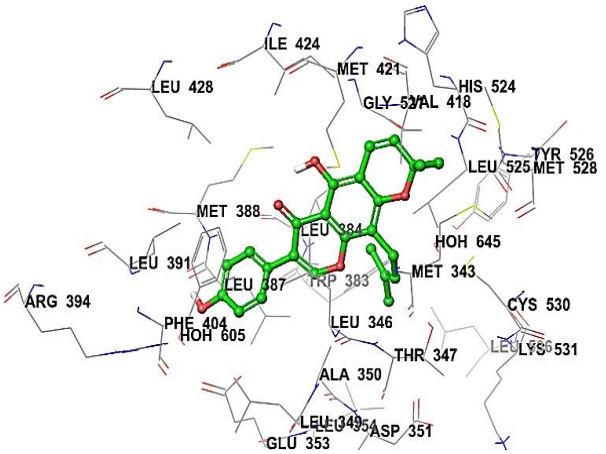
1a) Sissotrin (G Score: -11.91)



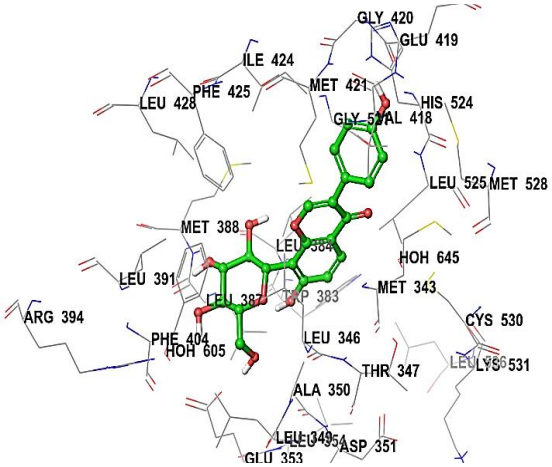
1b) Artocarpin (G Score: -11.68)



1c) Warangalone (G Score: -11.55)



1d) Puerarin (G Score: -11.43)



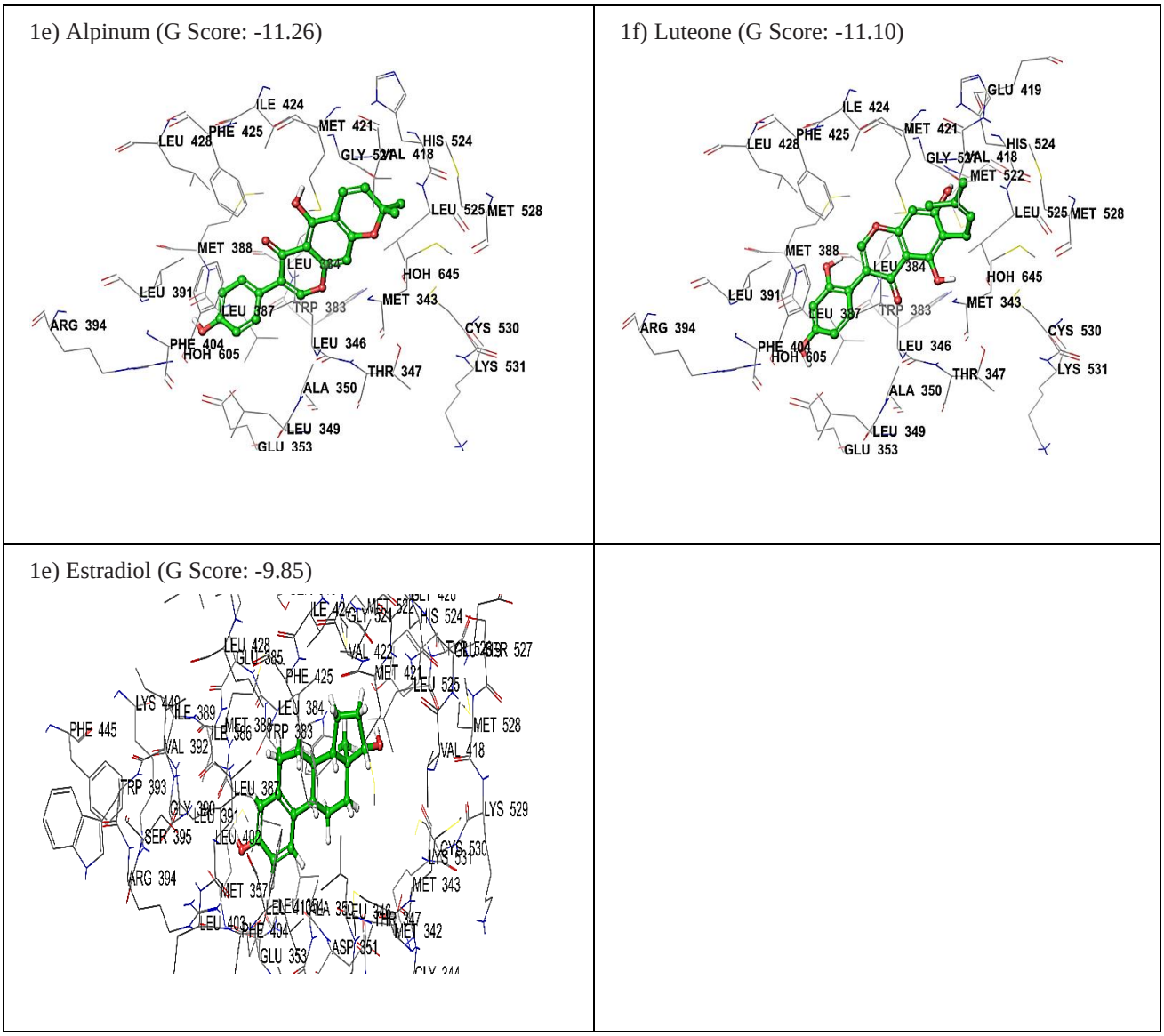
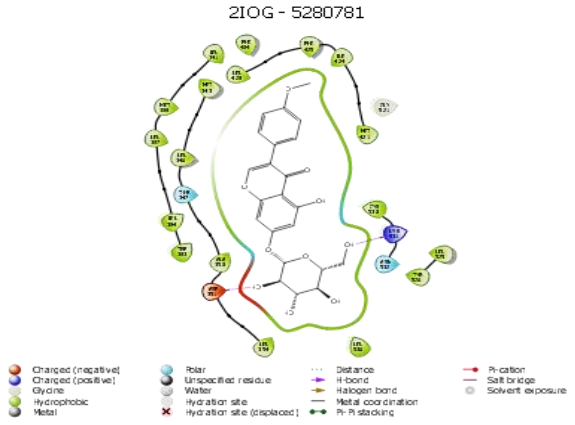
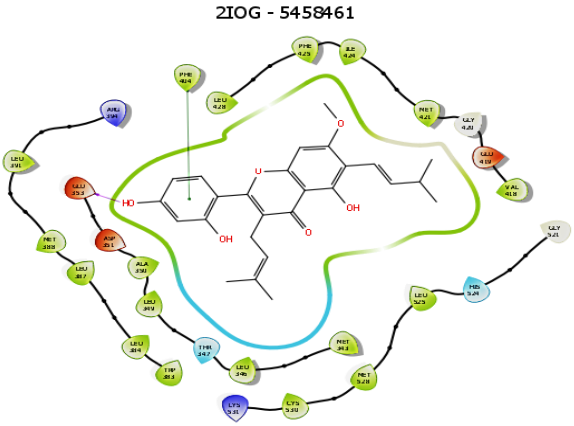


Figure 1: Best affinity mode of docked compounds with ERα (2IOG.pdb)

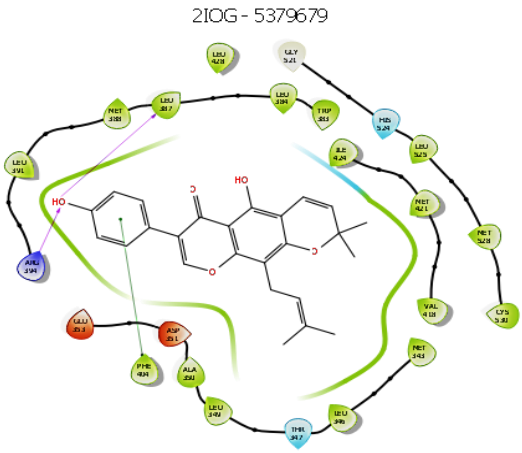
2a) Sissotrin(CID NO 5280781)(G Score: -11.91 kcal/mol)



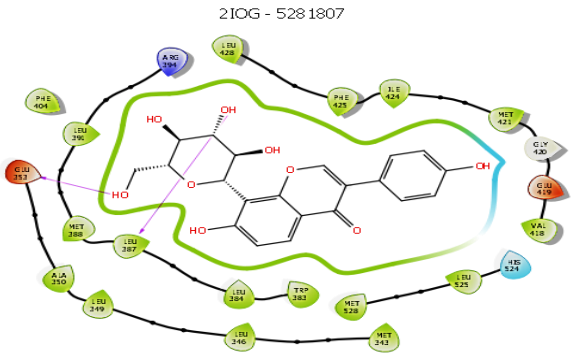
2b) Artocarpin(CID NO. 5458461) (G Score: -11.68 kcal/mol)



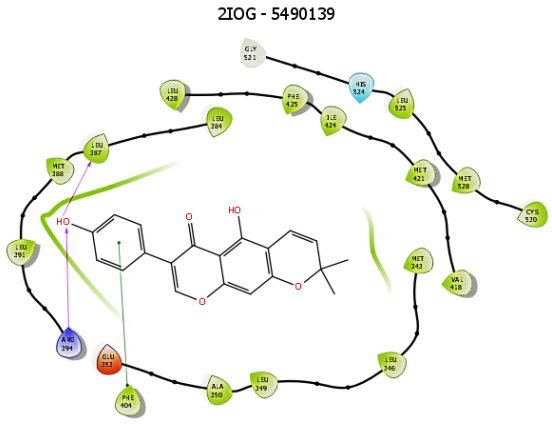
2c) Warangalone (CID NO. 5379679) (G Score: -11.55)



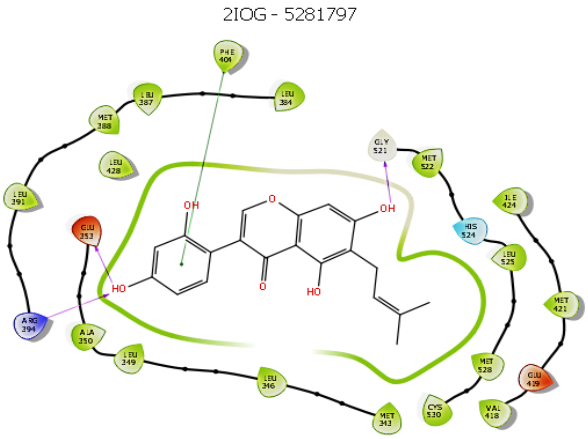
2d) Puerarin(CID NO. 5281807) (G Score: -11.43)



2e) Alpinum (CID NO. 5490139) (G Score: -11.26)



2f) Luteone (CID NO. 5281797) (G Score: -11.10)



2g) Estradiol (CID NO. 577) (G Score: -9.85)

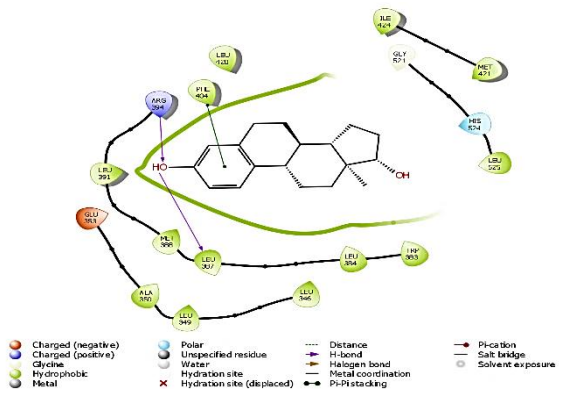


Figure 2: 2D Ligand interaction diagram of compounds with ERα(2IOG.pdb)

Table 2: Evaluation of ADME parameters of the naturally occurring isoflavones by Quikprop molecular docking suite.

S.No	Compound	QP log Po/w	QP log HERG	QPP Caco	QP log BB	QPP MDCK	QP log Kp	QP log Khsa	PHOA
	Albanin A	2.374	-4.845	81.403	-1.798	32.876	-4.268	0.235	75.04
	Alpinum	3.645	-5.657	662.354	-0.797	316.935	-2.42	0.555	100
	Artocarpin	5.38	-6.043	407.148	-1.536	187.3	-2.789	1.198	92.198
	Bavachinin	4.166	-5.555	996.492	-0.73	492.834	-2.297	0.713	100
	Biochanin A	2.507	-5.061	542.986	-0.864	255.675	-2.601	0.075	90.571
	Bowdichione	0.941	-4.751	173.176	-1.25	74.344	-3.825	-0.62	72.52
	Cajanin	1.912	-4.989	240.555	-1.269	106.051	-3.286	-0.077	80.761
	Calycosin	1.911	-5.097	391.448	-1.013	179.506	-2.91	-0.106	84.542
	Caragiside A	0.798	-5.867	153.953	-2.023	65.465	-3.256	-0.686	70.768

	Chrysin	2.371	-5.26	376.409	-0.885	172.063	-2.924	0.135	86.925
	Cladrin	2.764	-5.252	1267.84	-0.547	639.368	-1.922	0.057	100
	Daidzein	1.753	-5.08	385.172	-0.911	176.397	-2.839	-0.147	83.49
	Dalparvone	2.131	-5.08	273.288	-1.336	121.731	-3.214	-0.036	83.036
	Datiscetin	1.123	-5.042	75.282	-1.675	30.213	-4.292	-0.204	67.109
	Ermanin	2.701	-5.154	516.121	-0.935	242.03	-2.782	0.168	91.315
	Flemiphilippinin	5.743	-5.807	244.615	-1.681	107.987	-3.098	1.413	90.362
	Formononetin	2.587	-5.143	1269.126	-0.45	640.068	-1.879	0.021	100
	Gancaonin L	2.575	-5.264	79.654	-1.989	32.113	-4.178	0.268	76.052
	Genistein	1.659	-5	164.801	-1.323	70.465	-3.562	-0.107	76.336
	Glabranin	4.002	-5.125	701.485	-0.795	337.22	-2.503	0.702	100
	Glabrone	2.856	-5.656	624.918	-0.823	297.618	-2.473	0.33	93.71
	Glisoflavone	2.948	-5.569	256.892	-1.562	113.857	-3.229	0.267	87.335
	Glycitein	2.005	-5.114	578.817	-0.841	273.959	-2.522	-0.125	88.132
	Icaritin	3.254	-4.769	245.991	-1.34	108.644	-3.4	0.465	88.794
	Iridin	0.075	-5.563	20.419	-3.234	7.374	-5.005	-0.788	11.959
	Irigenin	2.206	-4.778	321.609	-1.303	145.154	-3.122	-0.051	84.741
	Irilone	1.969	-4.566	525.665	-0.745	246.871	-2.762	-0.102	87.172

	Irisflorentin	3.04	-5.033	4881.563	-0.048	2745.369	-1.03	-0.274	100
	Isosakuranetin	2.467	-5.04	428.058	-0.934	197.719	-3.043	0.147	88.49
	Isowighteone	3.371	-5.566	273.245	-1.411	121.711	-3.092	0.464	90.294
	Koparin	1.386	-5.032	208.893	-1.34	91.048	-3.414	-0.251	76.586
	Lachnoisoflavone B	3.15	-5.078	561.544	-0.808	265.133	-2.734	0.386	94.6
	Licoflavanone	3.341	-5.521	215.697	-1.495	94.257	-3.529	0.542	88.282
	Luteone	2.772	-5.266	168.236	-1.63	72.054	-3.464	0.242	83.015
	Medicarpin	3.092	-4.483	2998.149	-0.02	1620.958	-1.493	0.204	100
	Morin hydrate	0.398	-4.935	22.839	-2.258	8.323	-5.351	-0.352	53.591
	Morusin	4.679	-5.885	349.936	-1.326	159.021	-3.017	1.025	100
	Neobavaisoflavone	3.455	-5.649	642.414	-0.956	306.634	-2.364	0.413	100
	Norartocarpetin	0.985	-5.041	48.038	-1.883	18.591	-4.727	-0.197	62.81
	Orobol	0.983	-4.913	58.918	-1.82	23.181	-4.459	-0.262	64.388
	Pinocembrin	2.353	-5.064	428.054	-0.83	197.717	-2.947	0.126	87.825
	Pinostrobin	3.062	-5.112	1413.396	-0.359	719.064	-1.986	0.173	100
	Pomiferin	4.423	-5.63	265.9	-1.441	118.179	-3.122	0.911	96.243
	Poriol	2.046	-4.969	199.78	-1.258	86.762	-3.753	0.1	80.103
	Pratensein	1.819	-5.014	167.464	-1.438	71.697	-3.633	-0.064	77.402

	Pratol	2.591	-5.331	885.581	-0.565	433.82	-2.288	0.103	94.863
	Prunetin	2.501	-5.067	543.551	-0.865	255.962	-2.6	0.072	90.545
	Psedobaptigenin	2.042	-4.54	1271.787	-0.329	641.519	-2.019	-0.159	94.463
	Puerarin	-0.529	-5.804	15.901	-2.875	5.627	-5.236	-0.731	32.39
	Sissotrin	0.374	-5.748	39.888	-2.604	15.206	-4.368	-0.664	57.785
	Steppogenin	0.98	-4.852	53.051	-1.835	20.696	-4.768	-0.191	63.554
	Tectoridin	-0.145	-5.767	16.305	-3.129	5.782	-5.091	-0.76	21.877
	Tectorigenin	1.905	-4.886	254.203	-1.22	112.569	-3.226	-0.086	81.149
	Vestitone	1.993	-4.785	690.505	-0.693	331.519	-2.635	-0.085	89.428
	Warangalone	5.247	-5.845	662.443	-0.993	316.981	-2.392	1.182	95.205

QP log Po/w: Predicted octanol/water partition coefficient (-2.0 to 6.5); QP log HERG: Predicted IC50 value for blockage of HERG K+ channels (acceptable range: concern below -5.0); QPP Caco: Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells is a model for the gut-blood barrier (<25-poor, >500-great); QP log BB : predicted brain/blood partition coefficient(-3 to 1.2); QPP MDCK: Predicted apparent MDCK cell permeability in nm/s, MDCK cells are considered to be a good mimic for the blood-brain barrier (< 25-poor, >500-great); QP log Kp: Predicted skin permeability, log Kp. (–8.0 to –1.0); QP log Khsa: Prediction of binding to human serum albumin (–1.5 to 1.5); Percent Human Oral Absorption (PHOA): Predicted human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with Human Oral Absorption, as both measure the same property (>80% is high, <25% is poor).

Table 3: Binding free energy calculation using prime/MM-GBSA approach

S. NO	Ligand	ΔG Bind	ΔG Bind Coulomb	ΔG Bind Covalent	ΔG Bind H bond	ΔG Bind Lipo	ΔG Bind vdW
	Sissotrin	-67.17	26.08	5.04	1.93	-26.88	-60.87
	Artocarpin	-102.32	16.84	9.02	-0.11	-32.78	-84.40
	Warangalone	-70.81	53.87	-10.80	4.78	-34.12	-57.61

	Puerarin	-56.03	46.44	-4.93	2.71	-28.35	-58.14
	Alpinum	-68.06	42.70	-10.44	4.94	-32.75	-53.25
	Luteone	-57.51	37.40	-4.99	4.73	-23.91	-54.51
	Gancaonin L	-89.42	33.16	-2.40	2.37	-38.28	-80.22
	Neobavaisoflavone	-73.41	41.22	-7.46	2.95	-32.29	-63.86
	Orobol	-88.29	15.67	-15.03	2.15	-26.99	-54.76
	Lachnoisoflavon B	-61.59	70.36	-19.06	4.06	-21.41	-57.76
	Glabrone	-33.47	95.95	-9.84	5.83	-23.03	-50.27
	Dalparvone	-74.84	3.13	1.86	0.63	-25.58	-57.98
	Isowighteone	-60.59	59.13	-5.02	4.79	-28.87	-68.42
	Bavachinin	-81.69	39.92	-4.56	3.47	-35.90	-73.14
	Pomiferin	-68.58	53.24	-4.16	2.60	-35.87	-67.23
	Glabranin	-29.03	90.13	-8.74	6.19	-23.94	-44.82
	Koparin	-77.27	7.29	-8.78	1.07	-25.23	-45.19
	Albanin A	-77.87	13.17	-11.80	3.68	-20.28	-59.34
	Iridin	-57.39	57.41	-6.05	1.22	-29.67	-70.54
	Irigenin	-60.65	73.42	-17.75	4.94	-29.19	-61.92
	Calycosin	-72.94	43.67	-13.43	3.35	-27.58	-54.25
	Irisflorentin	-53.44	72.77	-6.14	4.96	-33.37	-46.42
	Morusin	-56.18	46.93	-2.35	1.88	-24.50	-65.72
	Genistein	-94.22	13.42	-20.25	1.72	-28.38	-50.22
	Biochanin A	-64.71	23.36	-7.28	2.05	-22.08	-46.78
	Tectorigenin	-101.95	17.49	-18.95	1.52	-32.81	-63.9
	Pinostrobin	-55.79	41.27	-2.78	2.84	-26.06	-66.92
	Pratensein	-73.30	33.50	-11.50	3.02	-24.28	-51.59
	Licoflavanone	-60.23	35.22	-17.07	4.19	-18.16	-57.85

	Formononetin	-69.37	35.69	-13.10	2.73	-28.27	-44.09
	Poriol	-19.26	59.66	8.22	4.05	-22.74	-44.21
	Irilone	-68.23	67.44	-10.03	2.09	-35.49	-65.52
	Caragiside A	-67.41	32.74	-8.43	2.61	-34.06	-72.39
	Morin hydrate	-39.28	27.30	-5.20	0.65	-12.07	-46.55
	Glisoflavone	-69.66	58.15	-17.98	6.17	-31.23	-62.82
	Pseudobaptigenin	-44.91	56.23	-11.53	4.57	-23.74	-32.68
	Cajanin	-70.44	34.92	-15.14	3.53	-19.20	-51.17
	Daidzein	-83.37	-1.07	-2.98	0.68	-26.81	-47.86
	Glycitein	-78.57	48.44	-5.39	5.34	-41.89	-63.75
	Cladrin	-66.29	74.40	-19.23	4.22	-29.19	-60.96
	Vestitone	-93.07	19.93	-13.51	1.39	-26.26	-65.60
	Datiscetin	-41.21	31.79	-5.57	2.91	-22.12	-42.99
	Prunetin	-60.82	27.62	-7.28	2.72	-30.68	-51.96
	Chrysin	-61.87	17.19	-10.34	2.78	-25.36	-54.35
	Ermanin	-40.26	85.72	-8.65	5.59	-29.05	-62.35
	Pratol	-49.70	74.08	-11.04	5.87	-28.25	-46.89
	Icaritin	-67.02	21.52	-3.79	1.44	-27.54	-64.52
	Bowdichione	-65.87	13.05	-6.84	1.84	-19.04	-51.93
	Tectoridin	-34.83	76.22	1.561	4.59	-25.33	-50.41
	Isosakuranetin	-56.80	70.48	-17.46	2.67	-18.09	-67.31
	Steppogenin	-45.38	30.04	-5.57	0.57	-6.695	-48.76
	Pinocembrin	-63.40	14.90	-11.75	1.01	-16.37	-46.47
	Norartocarpetin	-47.04	25.90	-4.34	0.81	-14.76	-55.99
	Medicarpin	-47.81	23.12	13.42	2.99	-34.77	-66.10

Acknowledgments

Authors wish to thank AICTE and DST-FIST, New Delhi for their funding and infrastructure support to Department of Pharmacology, JSS College of Pharmacy, Ooty, JSS Academy of Higher Education and Research (JSSAHER), P.B. 20, Rocklands, Ooty-643001, Tamilnadu, India.

REFERENCES

1. McNamara, K.; Alzubaidi, H.; Jackson, J.K. Cardiovascular disease as a leading cause of death: How are pharmacists getting involved? Integr. Pharm. Res. Pract.,2019; 8:1-11.
2. Shisan Xu, Fangjing Xie, Li Tian et al., Estrogen accelerates heart regeneration by promoting the inflammatory response in zebrafish. Journal of Endocrinology. 2020; 245:39-51.
3. Si Wan Lei, Guozhen Cui, George Pak Heng Leung et al., Icaritin protects against oxidative stress-induced injury in cardiac H9c2 cells via Akt/Nrf2/HO-1 and calcium signalling pathways. Journal of functional foods. 2015; 18:213-223.
4. R. Lakshmi Sundaram and Hannah R. Vasanthi. Dalspinin isolated from Spermacoce hispida (Linn.) protects H9c2 cardiomyocytes from hypoxic injury by modulating oxidative stress and apoptosis. Journal of Ethnopharmacology.2019;241:111962
5. Ramachandran, Vadivelan & Bairi, Raju & R, Kalirajan & Elumalai, Manogaran. Isoflavones Protective Mechanisms Against Cardiovascular Diseases. Int. J. Res. Pharm. Sci., 2020;11(3): 4670-4675.
6. Bin Liu, Jingzhi Zhang, Weihua Liu, Ningning Liu, Xiuquiong Fu, Hiuyee Kwan, Shaojun Liu, Benrong Liu, Shuangwei Zhang, Zhiling Yu, Shiming Liu. Calycosin inhibits oxidative stress-induced cardiomyocyte apoptosis via activating estrogen receptor-α/β. Bioorganic&Medicinal Chemistry Letters. 2016; 26:181–185
7. Brzezinski, A and Debi, A.Phytoestrogens: The "natural" selective estrogen receptor modulators? European Journal of Obstetrics&Gynecoogyl and Reproductive Biology. 1999;85: 47–51.
8. Anne M. Deschamps, Elizabeth Murphy and Junhui Sun. Estrogen Receptor Activation and Cardioprotection in Ischemia Reperfusion Injury. Trends Cardiovasc Med. 2010; 20(3):73–78.
9. Gencel, V.B.; Benjamin, M.M.; Bahou, S.N.; Khalil, R.A.Vascular effects of phytoestrogens and alternative menopausal hormone therapy in cardiovascular disease. Mini Rev Med Chem. 2012; 12:149–174.
10. Dworatzek E, Mahmoodzadeh S, Schubert C, Westphal C, Leber J, Kusch A, et al. Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor beta. Cardiovascular Research. 2014; 102:418–28.
11. Irsik DL, Carmines PK, Lane PH. Classical estrogen receptors and ER alpha splice variants in the mouse. PLoS ONE. 2013;8: e70926.
12. Lizotte E, Grandy SA, Tremblay A, Allen BG, Fiset C. Expression, distribution and regulation of sex steroid hormone receptors in mouse heart. Cell Physiol Biochem. 2009; 23:75–86.
13. Mahmoodzadeh S, Eder S, Nordmeyer J, Ehler E, Huber O, Martus P, et al. Estrogen receptor alpha up-regulation and redistribution in human heart failure. FASEB J. 2006; 20:926–34.
14. Nordmeyer J, Eder S, Mahmoodzadeh S, Martus P, Fielitz J, Bass J, et al. Upregulation of myocardial estrogen receptors in human aortic stenosis. Circulation. 2004; 110:3270–5.
15. Sara Menazza and Elizabeth Murphy. The Expanding Complexity of Estrogen Receptor Signaling in the Cardiovascular System. Circ Res. 2016; 118:994-1007.
16. NinadV. Puranik, Pratibha Srivastava, Gaurav Bhatt, Dixcy Jaba Sheeba John Mary, Anil M. Limaye & Jayanthi Sivaraman. Determination and analysis of agonist and antagonist potential of naturally occurring favonoids for estrogen receptor (ERα) by various parameters and molecular modelling approach. Scientific Reports.2019; 9:7450
17. Saravanan, G.; Ponmurugan, P.; Sathiyavathi, M.; Vadivukkarasi, S.; Sengottuvelu, S. Cardioprotective activity of Amaranthus viridis Linn: effect on serum marker enzymes,cardiac troponin and antioxidant system in experimental myocardial infarcted rats. Int. J. Cardiol.2013;165(3):494-498.
18. Maria Teresa Viggiani, Polimeno, L., Leo, A. D., Barone, M. Phytoestrogens: dietary intake, bioavailability, and protective mechanisms against colorectal neoproliferative lesions. Nutrients. 2019;11(8).
19. Sangeethadevi Govindasami, Veera Venkata Sathibabu Uddandrao, Nivedha Raveendran and Vadivukkarasi Sasikumar.Therapeutic Potential of Biochanin-A Against Isoproterenol-Induced Myocardial Infarction in Rats. Cardiovascular & Hematological Agents in Medicinal Chemistry. 2020;18: 31-36
20. Lengauer T, Rarey M. Computational methods for biomolecular docking. Current opinion in structural biology. 1996 Jun 1;6(3):402-6.
21. Alex. M and Nixon. R Docking Studies on Anticancer Drugs for Breast Cancer using Hex. Proceedings of the International Multi Conference of Engineers and Computer Scientists. 2009; 2:18 - 20.
22. Daniel, S and Bert, L., Ligand docking & binding site analysis with PyMOL and Auto Dock/vina. Journal of Computer Aided Molecular Design. 2010;24: 417-422.
23. Kalirajan, R., Gowramma, B., Jubie, S. and Sankar, S. 'Molecular docking studies and in-silico ADMET screening of some novel heterocyclic substituted 9-anilinoacridines as topoisomerase II inhibitors. JSM Chem., 2017;5:1039–1044.