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α -Selinene from *Syzygium aqueum* against Aromatase p450 in Breast Carcinoma of Postmenopausal Women: in Silico Study

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Abstract

Breast cancer is the most diagnosed and prevalent cancer in women both in developed and developing countries, this constitutes a threat to the society and world economic stability. *In situ* carcinoma and invasive carcinoma are the broad rubric of breast cancer. In established breast carcinoma, about 80% depends on the gird of estrogen hormone for growth. Hence, the down-regulation of aromatase activities has been one of the strategies used in the treatment of breast-related carcinomas.

This study explores S*yzygium aqueum* for the best drug-gable compound via computation tools. Seventy-one compounds were obtained from S*yzygium aqueum* plant which was retrieved from works of literature and were docked into the active site of aromatase p450 for their antagonistic effects. A-selinene, the lead compound with the binding energy of -8.5 kcal/mol was obtained using PyRx, autodock vina tools used in the molecular docking to obtain the docking scores. To ensure and validate that the right target was used, the FASTA sequence of the crystalline structure of aromatase p450 was blast on the chembl database.

Spearman rank correlation coefficient graph was plotted on graphpad prism 6 to obtain a strong correlation (R^2) value of 0.77 between the dockings results of the CHEMBL'S compounds and their corresponding experimentally generated IC50 results. These results explain why α-selinene should be considered a potential antagonist of aromatase p450 in postmenopausal women suffering from breast carcinomas.

Keywords: Aromatase p450; α-Selinene; Breast cancer; CYP19A1 gene; NADPH cytochrome p450 reductase; Androgens; Estrogens

Introduction

Cancer is a malignant disease condition emerging from uncontrollable cell division in the body to form mass of tissues. The cells have the capacity to metastasize; that is infiltrate adjacent or distant site in the body where they proliferate uncontrollably thus, causing significant mortality and morbidity [1].

According to the world health organization, 55% of women worldwide are overweight or obese [2]. Breast cancer is the most diagnosed cancer in women in developed and developing countries. Breast cancer in female is the most prevalent cancer in developing and developed countries; hence constitute a treat to the society and world economic stability. In situ carcinoma and invasive carcinoma are the broad rubric of breast cancer. Carcinoma *in situ* is further subdivided as either ductal carcinoma *in situ* (DCIS) or lobular cancer *in situ* (LCIS). While the two categories of DCIS are; comedocarcinoma and non-cosmedocarcinoma. In addition to this, the major invasive tumor types include invasive lobular (ILC) or invasive ductal carcinoma (IDC) which accounts for 70-80% of all invasive lesions [3-6].

DNA microarrays analysis sub-classified breast cancer into, luminal a: which is positive for ER, negative for HER2, low Ki-67 protein and high PR. Luminal b: which is positive for ER, negative for HER2, and either Ki-67 protein high or PR low, basal-like breast cancer: this lacks expression of the molecular targets that confer responsiveness to highly effective targeted therapies such as Tamoxifen and Aromatase inhibitors), Triple-negative breast cancer (TNBC) (ER-, PR-, and HER2-negative tumors), HER2+: (ERBB2+) has amplified HER2/neu. HER2-positive cancer is diagnosed in 10%–20% of breast cancer patients, which is particularly aggressive and more likely to spread rapidly than other types of breast cancer, claudin low (often TN, but distinct in that, there is low expression of cell–cell junction proteins including e-cadherin and are commonly infiltrated with lymphocytes) [7,8].

In established breast carcinoma, about 80%, depends on accouter of estrogen hormone for growth, hence, estrogen receptor positive (ER)

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carcinoma. Estrogens pioneer neoplastic growth via binding to their receptors in the tumor Figure1. In premenopausal women, the source of estrogen is the ovaries, while the skin, adipose tissue and the breast are the main source of estrogen in post-menopausal women. Hence, hormone therapy; that's the down-regulation of aromatase activities as been one of the strategies used in the treatment of breast related carcinomas [9,10].

Human aromatase enzyme, located in the endoplasmic reticulum, a member of the cytochrome p450 family and translational product of the CYP19A1 gene, located on chromosome 15 [11,12], a key enzyme required for the biosynthesis of estrogens. Aromatase is an heterodimer enzyme, in that it is made up of a ubiquitous NADPH cytochrome p450 reductase and cytochrome 450 aromatase; whose catalytic portion contains a heme group and a steroid binding site [13]. It catalyzes the rate-limiting step and the aromatization of androgens to estrogens Figure 2. The bimolecular reaction of aromatase is carried out in three oxidation reactions of the androstenedione a ring, in which each reaction utilize a molecule of both oxygen and NADPH. The first two reaction steps are common to p450 cytochrome proteins, while the third is unique to aromatase [14]. Carcinomas of the breast have been shown to express aromatase, which consequently leads to higher levels of estrogens than non-cancerous cells and the core reason why aromatase generates a high level of interest for the treatment of breast cancer [15].

The standard treatments for postmenopausal women with hormone receptor-positive breast cancer are the ALs. Been effective treatment for hormone receptor-positive breast cancer, their benefit is often circumscribed by emergence and interference of resistance which occurs in adjuvant setting and in metastatic breast cancer Figure 3. The two distinct pathways involved in resistance include ER signalling and growth factor receptor pathways. Carcinomas of the breast that either expresses ER or PR are known to be non-respondent to endocrinotherapeutic effects of AIs which is other way round for patients with higher levels of ER or PR level [16-18].

Water jambu (*Syzygium aqueum*), ethnomedically, water jambu plant parts have varied applications which includes, antibiotic activity, antifissures tongue, itching reliever, antiturgescence, antioxidant, antityrosinase, anticellulite activities, lipolytics, antihyperglycemic (α-glucosidase, α-amylases, advanced glycation end products (AGEs) and aldose reductase (AR) inhibitory activities). *Syzygium aqueum* aldose reductase inhibitory activity has been reported to reduce

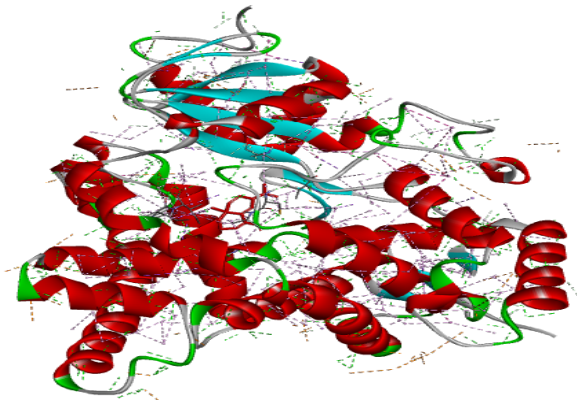

Figure 1: interactions of α-selinene (red sticks) within the binding pocket.

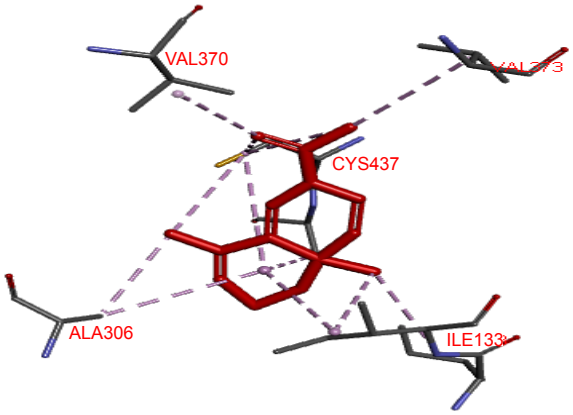

Figure 2: Alky interactions of α-selinene (red sticks) within the binding pocket.

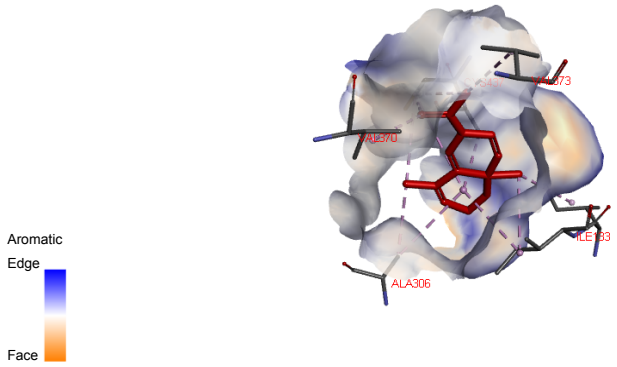

Figure 3: Aromatic interactions of α-selinene (red sticks) within the binding pocket.

microvascular complication of diabetes which includes retinopathy, nephropathy, neuropathy and cataracts [19-23].

Irreversible steroidal inhibitors (exemestane and formestane) and non-steroidal inhibitors (anastrozole and letrozole) are the two types of aromatase inhibitors approved for the treatment of hormonally-responsive breast cancer in postmenopausal women, since they had been proven to be superior to tamoxifene known as a representative of selective estrogen receptor modulators (SERMs). Pharmacotherapies (AIs), with distinct pharmacokinetics and pharmacodynamics properties, and other orthodox methods used in the treatment of postmenopausal ER+ breast cancer as lead to varied pathophysiological contradictions such as; osteoporosis, osteopenia, arthralgia, myalgia, hot flashes, night sweats and vaginal dryness. Hence, a need for the discovery of more potent, efficient, cost-effective active principles of plant origin with little or no side effects which are capable to alleviate ais complications [24-29].

Materials and Methods

Ligand selection and preparation

Seventy-one (71) phytochemical of S*yzygium aqueum* plant were retrieved from pubchem compound database (https://pubchem.ncbi.

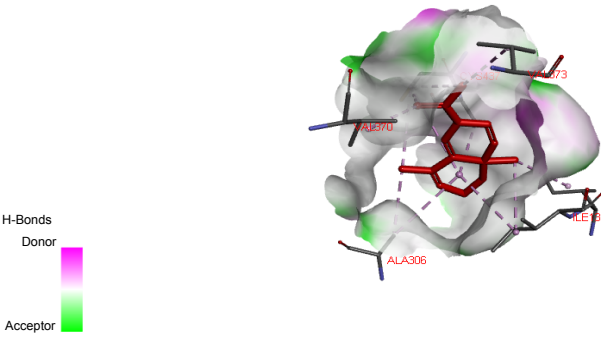

Figure 4: Hydrogen-Bond interactions of α-selinene (red sticks) within the binding pocket.

nlm.nih.gov). The MOL SDF format of these ligands were converted to PDBQT file using PyRx tool to generate atomic coordinates and energy was minimized by using the optimization algorithm at force field set at mmff (required) on PyRx Figure 4.

Accession and preparation of the target protein

The three-dimension crystal structure of aromatase p450 (PDB: 5jkv) in complex with a co-crystallized ligand was retrieved from RCSB PDB (http://www.rcsb.org/pdb/home/home.do) [8]. Pymol tool was used to clean the protein by removing non-essential water molecules and all heteroatoms that complex with the protein. The grid coordinate around the active site was revealed by the extraction of the ligand.

Molecular docking using PyRx

For our analysis we used the PyRx, autodock vina exhaustive search docking function. After the minimization process, the grid box resolution was centered at 83.4520 × 50.1752 × 46.4060 along the x, y and z axes respectively at grid dimension of 25 × 25 × 25 Å to define the binding site. The co-crystallized ligand which serves as the standard was first docked within the binding site of aromatase p450 and the resulting interaction was compared with that of α-selinene into the similar active sites using the same grid box dimension.

Validation of docking results

Validation of the docking score obtained was carried out by blasting the FASTA sequence of the crystalline structure of aromatase p450 which was obtained from protein data bank unto the online available CHEMBL database (www.ebi.ac.uk/chembl/). The bioactivity generated by the database, having an inhibition of 959 and IC50 value of 2617, was downloaded in txt format. The bioactivity was sorted out; missing or misplaced data were removed. Only 28 of the total 2617 drug-like compounds were recovered. The compiled compounds were split and converted to 2d (in SDF format) by Datawarrior software (version 2) and converted to PDBQT format by PyRx tool. The ligands were docked into the binding domain of aromatase p450 using PyRx Autodock Vina scoring function. A correlation coefficient graph was plotted between the docking scores of the 28 compounds generated and their corresponding PCHEMBL_VALUE (experimentally determined) values. Spearman rank correlation co efficient graph was plotted on graphpad prism 6 to obtain a strong correlation (R^2) value between the dockings results of the CHEMBL's compounds and their corresponding experimentally generated IC50 results Figure 5.

Results and Discussion

CYP19A1 gene located on chromosome 15 synthesizes the heterodimer aromatase p450, a key enzyme required for the biosynthesis

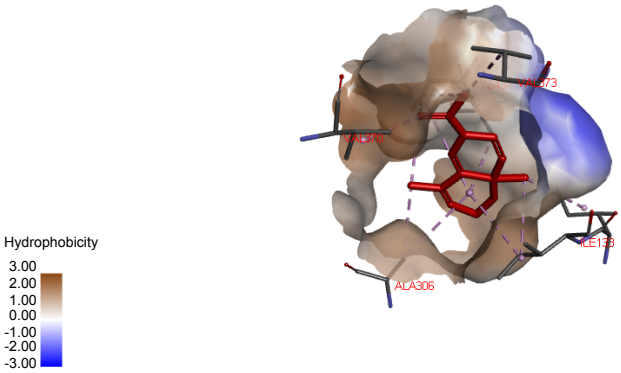

Figure 5: Hydrophobic interactions of α-selinene (red sticks) within the binding pocket.

Ligand	Binding Energy	Binding Affinity	Rmsd/ub	Rmsd/lb
Standard_0	E=373.82	-7.7	0	0
Compound_1	E=13.90	-5.9	0	0
Compound_2	E=97.17	-4.2	0	0
Compound_3	E=25.69	-6.4	0	0
Compound_4	E=67.31	-5.2	0	0
Compound_5	E=3.07	-4.5	0	0
Compound_6	E=-3.74	-4.3	0	0
Compound_7	E=223.31	-5.8	0	0
Compound_8	E=49.72	-3.8	0	0
Compound_9	E=677.22	-5	0	0
Compound_10	E=19.61	-5.8	0	0
Compound_11	E=148.71	-6.2	0	0
Compound_12	E=140.00	-6	0	0
Compound_13	E=140.20	-6.1	0	0
Compound_14	E=22.68	-6.1	0	0
Compound_15	E=14.00	-5.5	0	0
Compound_16	E=22.39	-5	0	0
Compound_17	E=18.92	-6.2	0	0
Compound_18	E=17.62	-5.5	0	0
Compound_19	E=-2.31	-4.7	0	0
Compound_20	E=-2.38	-4.7	0	0
Compound_21	E=-30.24	-4	0	0
Compound_22	E=11.80	-4.9	0	0
Compound_23	E=-0.15	-5.3	0	0
Compound_24	E=13.24	-5.2	0	0
Compound_25	E=-1.59	-5	0	0
Compound_26	E=18.23	-4.3	0	0
Compound_27	E=648.80	-6	0	0
Compound_28	E=41.86	-4.1	0	0
Compound_29	E=140.67	-6.1	0	0
Compound_30	E=43.08	-4.1	0	0
Compound_31	E=-0.11	-3.4	0	0
Compound_33	E=30.12	-5.2	0	0
Compound_32	E=315.62	-6.5	0	0

Compound_34	E=70.37	-6.2	0	0
Compound_35	E=31.40	-5.9	0	0
Compound_36	E=17.42	-4.3	0	0
Compound_37	E=8.84	-4.8	0	0
Compound_38	E=385.28	-6.3	0	0
Compound_39	E=1161.81	-8.1	0	0
Compound_40	E=204.01	-6.2	0	0
Compound_41	E=138.34	-6.1	0	0
Compound_42	E=167.47	-7.9	0	0
Compound_43	E=1308.26	-8.4	0	0
Compound_44	E=2.61	-4.9	0	0
Compound_45	E=-4.35	-5.7	0	0
Compound_46	E=15.11	-5.4	0	0
Compound_47	E=15.60	-4.7	0	0
Compound_48	E=5.23	-5	0	0
Compound_49	E=2.69	-5	0	0
Compound_50	E=203.42	-8.4	0	0
Compound_51	E=44.06	-6.9	0	0
Compound_52	E=30.91	-5.7	0	0
Compound_53	E=2.68	-5.2	0	0
Compound_54	E=1.12	-5	0	0
Compound_55	E=2.28	-5.7	0	0
Compound_56	E=-1.59	-5	0	0
Compound_57	E=-1.17	-4.8	0	0
Compound_58	E=1.09	-5.3	0	0
Compound_59	E=12.43	-4.9	0	0
Compound_60	E=192.35	-6.1	0	0
Compound_61	E=29.96	-5	0	0
Compound_62	E=16.37	-5.4	0	0
Compound_63	E=62.20	-7	0	0
Compound_64	E=404.73	-6.8	0	0
Compound_65	E=385.28	-6.3	0	0
Compound_66	E=159.61	-6.1	0	0
Compound_68	E=93.38	-6.5	0	0
Compound_69	E=70.37	-6.2	0	0
Compound_70	E=318.85	-8.5	0	0
Compound_71	E=884.29	-8.1	0	0

Table 1: Interaction table showing the various chemical interactions of α-selinene within the binding pocket (Viewed on Discovery studio Visualizer).

of estrogens. It catalyzes the rate-limiting step and the aromatization of androgens to estrogens Table 1. Carcinomas of the breast have been shown to express aromatase, which consequently leads to higher levels of estrogens than non-cancerous cells and the core reason why aromatase generates a high level of interest for the treatment of breast cancer.

In the present studies, seventy-one (71) phytocompounds from *Syzygium aqueum* plant were docked into the binding pocket of aromatase p450 for their antagonistic properties. A-selinene, the lead compound with the binding energy of -8.5 kcal/mol compared to the standard (-7.7 kcal/mol). The drug-likeness properties of α-selinene violated none of the lipinski's rule of five, and this describes the binding potential and bioavailability of α-selinene.

The highest binging energy attributed to α-selinene is believed to be due to the strong interaction at the actives site, which includes; electrostatic, hydrogen, and hydrophobic interaction (Table 2 Figure 6).

1) Thirty-six hydrogen bond interactions which involves; ARG 115, 145, 375, 435, ILE 133, 132, ARG 435, GLU 302, TRP 141, GLY 436, 439, PHE 148, 430, LEU 152, ALA 306, 307, GLU 302, 439, THR 310, CYS 437

2) Fifty-four hydrophobic interactions which involves; ILE 132, 133, PHE 148, 430, ARG 145, 435, ALA 306, 438, CYS 437, LEU 162, 437, VAL 373, 370, TRP 141, MET 303

3) Four electrostatic interactions which involves; ARG 415 and TRP 141

While the standard as the following interaction at the binding pocket which involves;

4) Six hydrogen interactions which involves MET 311, ALA 307, 443, CYS 437, PHE 430 and GLY 439

5) Twenty-two interaction hydrophobic interactions which involves ALA 306, 307, 443, MET 311, CYC 437, VAL 370, PHE 430 (Table 3).

The highest binding energy (-8.5 kcal/mol) attributed to α-selinene is believed to be as a result of the extensive high number of hydrophobic interaction of α-selinene with the pocket binding site. The average number of hydrophobic atoms in marketed drugs is 16, with one or two donors and three to four acceptors. This depicts the importance of hydrophobic interaction in the design of drugs. This further explains the high binding affinity of between target-drug interfaces. The dependability of our docking scores was further confirmed using the CHEMBL database. The compounds obtained from aromatase p450 FASTA sequence blast were docked into the binding pocket of aromatase p450; a strong correlation coefficient of 0.77 was obtained

S/N	Complex	Binding energy (From PyRx)	RMSD/UB[a]	RMSD/LB[b]
70	Alpha-Selinene (10856614)	-8.5	0	0
50	Caryophyllene (5281515)	-8.4	0	0
43	Beta-salinene (442393)	-8.4	0	0
39	Alpha-cabebene (88609)	-8.1	0	0
71	Bicyclogermacrene	-8.1	0	0
0	Standard	-7.7	0	0

RMSD/UB: Root mean square deviation/upper bond; RMSD/LB: Root mean square deviation/lower bond

Table 2: Interaction table showing α-Selinene of the five chemical compounds with highest bonding energy within the binding pocket (Viewed on Discovery studio Visualizer)

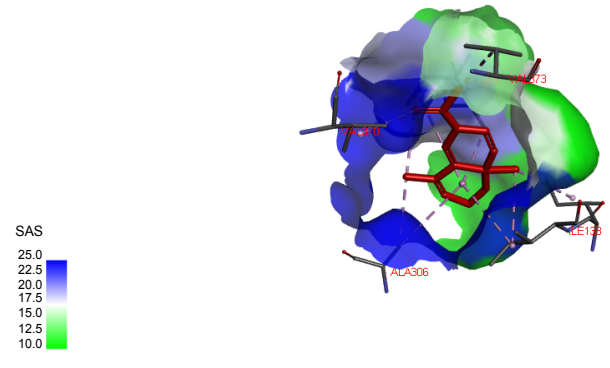

Figure 6: Solvent accessibility surface of the receptor and α-selinene interaction (red sticks) within the binding pocket.

Name	Category	Types
A:ARG115:HE - A:ILE133:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG115:HH21 - A:ARG435:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE132:HN - A:GLU302:OE1	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE133:HN - A:GLU302:OE1	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HN - A:TRP141:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HH11 - A:GLY436:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HH21 - A:GLY436:O	Hydrogen Bond	Conventional Hydrogen Bond
A:PHE148:HN - A:ARG145:O	Hydrogen Bond	Conventional Hydrogen Bond
A:LEU152:HN - A:PHE148:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ALA306:HN - A:GLU302:O	Hydrogen Bond	Conventional Hydrogen Bond
A:THR310:HN - A:ALA306:O	Hydrogen Bond	Conventional Hydrogen Bond
A:THR310:HN - A:ALA307:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG375:HH22 - A:ARG435:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG435:HH22 - A:ILE132:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG435:HH22 - A:ILE133:O	Hydrogen Bond	Conventional Hydrogen Bond
A:CYS437:HN - A:PHE430:O	Hydrogen Bond	Conventional Hydrogen Bond
A:GLY439:HN - A:CYS437:SG	Hydrogen Bond	Conventional Hydrogen Bond
A:TRP141:CD1 - A:ILE132:O	Hydrogen Bond	Carbon Hydrogen Bond
A:ARG435:NH1 - A:TRP141	Electrostatic	Pi-Cation
A:ARG435:NH1 - A:TRP141	Electrostatic	Pi-Cation
A:ILE132:CD1 - A:PHE148	Hydrophobic	Pi-Sigma
A:ILE133 - N:UNK1	Hydrophobic	Alkyl
A:ARG145 - A:ILE132	Hydrophobic	Alkyl
A:ALA306 - N:UNK1	Hydrophobic	Alkyl
A:ALA306 - N:UNK1:C	Hydrophobic	Alkyl
A:CYS437 - N:UNK1	Hydrophobic	Alkyl
A:ALA438 - A:LEU152	Hydrophobic	Alkyl
A:ALA438 - N:UNK1	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:ILE132	Hydrophobic	Alkyl
N:UNK1:C - A:ILE133	Hydrophobic	Alkyl
N:UNK1:C - A:VAL370	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:VAL373	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
A:TRP141 - A:ILE132	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG145	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG435	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG435	Hydrophobic	Pi-Alkyl
A:PHE148 - A:MET303	Hydrophobic	Pi-Alkyl
A:PHE430 - A:CYS437	Hydrophobic	Pi-Alkyl
A:ILE133 - N:UNK1	Hydrophobic	Alkyl
A:ALA306 - N:UNK1	Hydrophobic	Alkyl
A:ALA306 - N:UNK1:C	Hydrophobic	Alkyl
A:CYS437 - N:UNK1	Hydrophobic	Alkyl
A:ALA438 - N:UNK1	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:ILE132	Hydrophobic	Alkyl
N:UNK1:C - A:ILE133	Hydrophobic	Alkyl
N:UNK1:C - A:VAL370	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:VAL373	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
A:ARG115:HE - A:ILE133:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG115:HH21 - A:ARG435:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE132:HN - A:GLU302:OE1	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE133:HN - A:GLU302:OE1	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HN - A:TRP141:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HH11 - A:GLY436:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG145:HH21 - A:GLY436:O	Hydrogen Bond	Conventional Hydrogen Bond
A:PHE148:HN - A:ARG145:O	Hydrogen Bond	Conventional Hydrogen Bond
A:LEU152:HN - A:PHE148:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ALA306:HN - A:GLU302:O	Hydrogen Bond	Conventional Hydrogen Bond
A:THR310:HN - A:ALA306:O	Hydrogen Bond	Conventional Hydrogen Bond
A:THR310:HN - A:ALA307:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG375:HH22 - A:ARG435:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG435:HH22 - A:ILE132:O	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG435:HH22 - A:ILE133:O	Hydrogen Bond	Conventional Hydrogen Bond
A:CYS437:HN - A:PHE430:O	Hydrogen Bond	Conventional Hydrogen Bond
A:GLY439:HN - A:CYS437:SG	Hydrogen Bond	Conventional Hydrogen Bond
A:TRP141:CD1 - A:ILE132:O	Hydrogen Bond	Carbon Hydrogen Bond
A:ARG435:NH1 - A:TRP141	Electrostatic	Pi-Cation
A:ARG435:NH1 - A:TRP141	Electrostatic	Pi-Cation
A:ILE132:CD1 - A:PHE148	Hydrophobic	Pi-Sigma
A:ILE133 - N:UNK1	Hydrophobic	Alkyl
A:ARG145 - A:ILE132	Hydrophobic	Alkyl
A:ALA306 - N:UNK1	Hydrophobic	Alkyl
A:ALA306 - N:UNK1:C	Hydrophobic	Alkyl
A:CYS437 - N:UNK1	Hydrophobic	Alkyl
A:ALA438 - A:LEU152	Hydrophobic	Alkyl
A:ALA438 - N:UNK1	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:ILE132	Hydrophobic	Alkyl
N:UNK1:C - A:ILE133	Hydrophobic	Alkyl
N:UNK1:C - A:VAL370	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
N:UNK1:C - A:VAL373	Hydrophobic	Alkyl
N:UNK1:C - A:CYS437	Hydrophobic	Alkyl
A:TRP141 - A:ILE132	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG145	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG435	Hydrophobic	Pi-Alkyl
A:TRP141 - A:ARG435	Hydrophobic	Pi-Alkyl
A:PHE148 - A:MET303	Hydrophobic	Pi-Alkyl
A:PHE430 - A:CYS437	Hydrophobic	Pi-Alkyl

Table 3: Interaction table showing the various chemical interactions of α-Selinene within the binding pocket.

Name	Category	Types
A:MET311:HN - A:ALA307:O	Hydrogen Bond	Conventional Hydrogen Bond
A:CYS437:HN - A:PHE430:O	Hydrogen Bond	Conventional Hydrogen Bond
A:GLY439:HN - A:CYS437:SG	Hydrogen Bond	Conventional Hydrogen Bond
A:ALA443:HN - A:GLY439:O	Hydrogen Bond	Conventional Hydrogen Bond
A:GLY439:CA - N:ASD602:O	Hydrogen Bond	Carbon Hydrogen Bond
A:ALA306 - N:ASD602	Hydrophobic	Alkyl
A:ALA307 - A:MET311	Hydrophobic	Alkyl
A:ALA307 - N:ASD602:C	Hydrophobic	Alkyl
A:CYS437 - N:ASD602	Hydrophobic	Alkyl
A:CYS437 - N:ASD602	Hydrophobic	Alkyl
A:ALA443 - A:MET311	Hydrophobic	Alkyl
A:ALA443 - N:ASD602	Hydrophobic	Alkyl
A:ALA443 - N:ASD602	Hydrophobic	Alkyl
N:ASD602:C - A:VAL370	Hydrophobic	Alkyl
N:ASD602:C - A:MET311	Hydrophobic	Alkyl

A:PHE430 - A:CYS437	Hydrophobic	Pi-Alkyl
A:PHE430 - A:ALA443	Hydrophobic	Pi-Alkyl
A:PHE430 - N:ASD602	Hydrophobic	Pi-Alkyl
A:GLY439:CA - N:ASD602:O	Hydrogen Bond	Carbon Hydrogen Bond
A:ALA306 - N:ASD602	Hydrophobic	Alkyl
A:ALA307 - N:ASD602:C	Hydrophobic	Alkyl
A:CYS437 - N:ASD602	Hydrophobic	Alkyl
A:CYS437 - N:ASD602	Hydrophobic	Alkyl
A:ALA443 - N:ASD602	Hydrophobic	Alkyl
A:ALA443 - N:ASD602	Hydrophobic	Alkyl
N:ASD602:C - A:VAL370	Hydrophobic	Alkyl
N:ASD602:C - A:MET311	Hydrophobic	Alkyl
A:PHE430 - N:ASD602	Hydrophobic	Pi-Alkyl

Table 4: Interaction table showing the chemical interaction of the co-crystalized ligand within the binding pocket.

Lipinski's Rule of Drug Evaluation α-selinene	
MW<=500	204.357
Hacc<=10	0
Hdon<=5	0
Log P<=5	4.725
MR <=	66.743
TPSA<=140	0.0
Keys: MW=Molecular Weight, Hydrogen bond acceptor, Hydrogen bond donor, MR=Molar Refractivity, TPSA=Topological Polar Surface Area	

Table 5: Lipinski's drug-like properties of α-selinene: The rule describes drug candidate's pharmacokinetics in the human body which also including their absorption, distribution, metabolism, and excretion ("ADME") using an online server (http://www.scfbio-iitd.res.in/).

when the docking scores of the compounds generated were plotted against chembl's pchem values (experimentally determined IC50) as indicated in Table 4 Figure 7. The reliability of our docking scores was further validated using the online available CHEMBL database; the FASTA sequence of the crystal structure of aromatase p450 was blasted on www.ebi.ac.uk/chembl/. The compounds obtained from the search were docked into the binding site of the aromatase p450, a correlation coefficient graph plotted between the docking scores of the compounds generated and their corresponding CHEMBL'S Pchem values

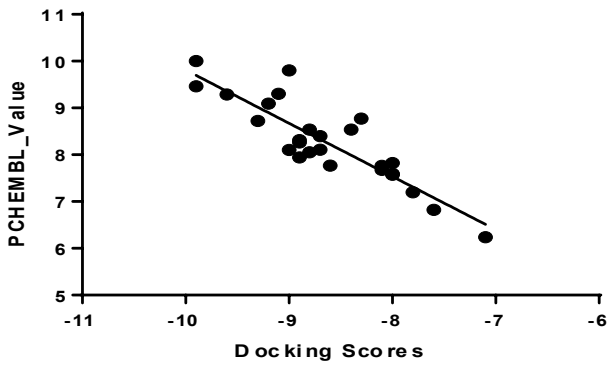

Figure 7: Correlation coefficient graph of docking scores of various antagonists of the aromatase p450 and their corresponding experimental IC50 (Pchembl values) values.

Predicted Physicochemical Properties of α-selinene						
LogS (Solubility)	**$LogD_{7.4}$** (Distribution Coefficient D)	**LogP** (Distribution Coefficient D)	LogS: Optimal: higher than -4 log mol/L $LogD_{7.4}$: 1 to 3: Solubility moderate; Permeability moderate; Metabolism low. LogP: LogP >3: poor aqueous solubility			
-5.89 log mol/L	2.954	4.725				
Predicted Selected Absorption Properties of α-selinene						
HIA (Human Intestinal Absorption)	**F** (20% Bioavailability)	**F** (30% Bioavailability)	≥30%: HIA+; <30%: HIA- ≥20%: F20+; <20%: F20- ≥30%: F30+; <30%: F30			
0.877*	0.711*	0.54*				
Predicted Selected Distribution Properties of α-selinene						
PPB (Plasma Protein Binding)	**VD** (Volume Distribution)	**BBB** (Blood–Brain Barrier)	0.07-0.7L/kg: Evenly distributed BB ratio >=0.1: BBB+; BB ratio <0.1: BBB			
75.9 %	0.42 L/kg	0.986*				
Predicted Selected Metabolism Properties of α-selinene						
P450 CYP3A4 inhibitor	P450 CYP3A4 substrate	P450 CYP2C9 inhibitor	P450 CYP2C9 substrate	P450 CYP2C19 inhibitor	P450 CYP2C19 substrate	
0.04*	0.659*	0.02*	0.45*	0.215*	0.64*	
Predicted Selected Elimination Properties of α-selinene						
$T_{1/2}$ (Half Life Time)	**CL** (Clearance Rate)	Range: >8h: high; 3h< Cl <8h: moderate; <3h: low Range: >15 mL/min/kg: high; 5mL/min/kg< Cl <15mL/min/kg: moderate; <5 mL/min/kg: low				
1.871 h	1.853mL/min/kg					
Predicted Selected Toxicity Properties of α-selinene						
H-HT (Human Hepatotoxicity)	**AMES** (Ames Mutagenicity)	**LD50** (LD50 of acute toxicity)	High-toxicity: 1~50 mg/kg; Toxicity: 51~500 mg/kg; low-toxicity: 501~5000 mg/kg [34].			
0.236*	0.108*	4058.724 mg/kg				
* Probability						

Table 6: ADMET Evaluation properties of α-selinene using online web-based server (http://admet.scbdd.com/)

(experimentally determined IC50). This portrays a strong correlation coefficient between docking scores and the experimentally derived ones. Hence making computation experiment replicable and docking scores using PyRx autodock vina algorithm is dependable (Table 5).

Conclusion

Docking studies and ADMET evaluation of α-selinene with aromatase p450 showed that the ligand is a drug-gable molecule which plays critical role in the antagonism of aromatase p450, hence should be consider as a potential agent in breast cancer therapy (Table 6).

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