Research Article

Molecular Docking Studies on Borapetol with Target Aromatase Related to Breast Cancer

Olusola Olalekan Elekofehinti*

Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

ABSTRACT

Aromatase, a cytochrome P450 enzyme complex present in breast tissues, plays a significant role in the biosynthesis of important endogenous estrogens**.** In hormone-dependent breast cancer, estrogen plays a significant role in the stimulation of breast cancer cell proliferation. Inhibition of aromatase has been put forward to be a therapeutic target for breast cancer. *In silico* predictions based on the crystal structure of aromatase was done. Active molecular docking studies using Autodock software revealed that borapetol is a novel inhibitor of aromatase with binding affinity of -9.4 kcal/mol compared to known aromatase inhibitor, anastrozole with binding affinity of -7.9 kcal/mol. The results showed that borapetol portrayed a better affinity to bind aromatase, by interacting at the catalytic site of enzyme. Hence, borapetol may be a potent lead compound for the treatment of breast cancer but further experimental and clinical confirmation is needed to prove it as valid drug.

Keywords: Cancer, Bioinformatics, *In silico*, Drug development, molecular docking, Borapetol.

INTRODUCTION

Cancer is a major public health problem globally. It is the second leading cause of death[1]. Cancer is a group of diseases characterized by abnormal (uncontrolled) growth and spread of abnormal cells. More than 100 types of cancer have been reported and their symptoms vary widely[2] but in Nigeria, the most common cancers are breast, cervix, prostate, liver, lungs and brain cancer[2].

Breast cancer is now the leading cause of cancer death among females and is a classical model of hormone-dependent malignancy. It is known that estrogens are involved in the growth and differentiation of the normal mammary gland and they intervene in the growth of the ductal branching, however, there is considerable evidence that estrogens are also mammary carcinogens[3].

Aromatase belongs to the cytochrome p450 family. This enzyme is localized in the endoplasmic reticulum of the cell[4]. Its primary function is to produce estrogens which are the key to pituitary, breast, and endometrial growth and development[5], Documented evidence in literature showed that the concentration of estrogens is twenty-fold higher in breast cancer tissues than in the circulating plasma, suggesting locally increased aromatase expression for estrogen biosynthesis near or within the cancerous tissues [6,7]. Inhibition of the aromatase enzyme has been shown to reduce estrogen production throughout the body to nearly undetectable levels and is proving to have significant affect on the development and progression of hormone-responsive breast cancers[8]. Treatment with aromatase inhibitors have been proposed as the solution to breast cancer in women.

Anastrozole, an aromatase inhibitor, is used with other treatments, such as surgery or radiation, to treat breast cancer in women. However, the side effect of this drug has been reported such as: weakness, headached, weight gain, diarrhea, loss of apetite, depression[9]. This calls for the search of a new and safer aromatase inhibitor especially from natural sources. Borapetol is a bioactive compound isolated from *Tinospora crispa* whose antidiabetic potential has been documented[10].

Bioinformatics tools have become very important to pinpoint the targets for different ligands. Many studies have indicated that computational approaches, such as structural bioinformatics[11],

molecular docking[12,13] , pharmacophore modeling[14] are best choice. Using bioinformatics tools we tried to evaluate whether borapetol, a bioactive compound from plant is a better ligand of the aromatase, the target protein related to breast cancer.

MATERIALS AND METHODS

Protein preparation and Generation of 3-D structure through homology modeling

The starting structure (PDB ID: 4GL7) required for docking was retrieved from the protein data bank repository (http: //www.rcsb.org). Prior to docking, water and ligand coordinates were deleted. Human aldose reductase "Fasta" file was downloaded from www.pubmed.org and used to model the starting structure of aromatase used in the current study. Homology modeling was done on Swiss Model Server (http://swissmodel.expasy.org). This requires one sequence of known 3D structure with significant similarity with the target sequence. The co-ordinate file of template from protein data bank (PDB ID: 4GL7) was used to model the 3D structure of aromatase.

Ligand Preparation for Docking

The structure of borapetol and anastrozole were built using Marvinsketch and optimized for docking studies. The optimized ligand molecules (borapetol and anastrozole) were docked into refined aromatase model using "LigandFit" in the AutoDock 4.2.

Molecular docking

Molecular Docking calculations were performed through BSP-SLIM and Autodock. The modeled structure of aromatase molecule and borapetol was loaded on BSP-SLIM server and Autodock/Vina and all the water molecules were removed prior to the upload. BSP-SLIM is known as a blind docking method, which primary uses the structural template match to identify putative ligand binding sites, followed by fine-tuning and ranking of ligand conformations in the binding sites through the SLIM-based shape and chemical feature comparisons[15].

Data analysis

Protein snapshots were taken using PYMOL.

RESULTS

Table 1: Showing binding energy of anasrozole with aromatase

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.9	0.000	0.000
2	-7.9	0.102	4.677
3	-7.8	1.863	5.695
4	-7.8	2.531	4.825
5	-7.8	2.519	4.841
6	-7.7	1.870	4.582
7	-7.6	1.963	5.996
8	-7.6	1.906	4.974
9	-7.6	1.250	4.577
10	-7.5	3.395	5.883

Table 2: Showing binding energy of borapetol with aromatase

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.4	0.000	0.000
2	-9.4	2.797	6.267
3	-9.3	0.712	1.002
4	-9.1	2.520	7.051
5	-8.9	1.851	7.269
6	-8.6	2.222	4.172
7	-8.6	2.848	6.355
8	-8.3	1.989	6.702
9	-8.3	2.757	5.067
10	-8.2	1.917	4.370

Figure 2: 3D structure of human aromatase

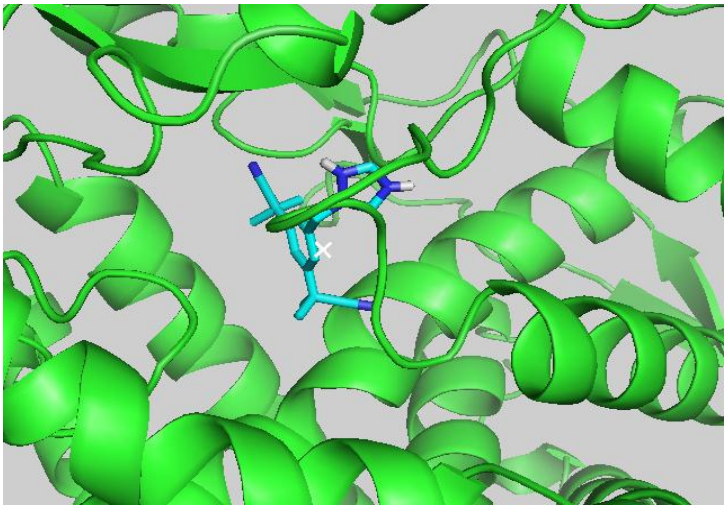

Figure 3: Binding pose/mode of aromatase with anastrozole with binding affinity of -7.9 kcal/mol

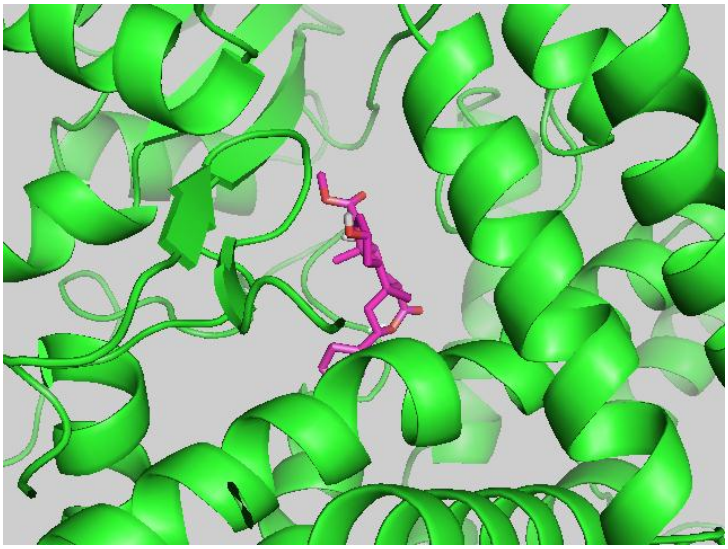

Figure 4: Binding pose/mode of aromatase with borapetol with binding affini of -9.4 kcal/mol

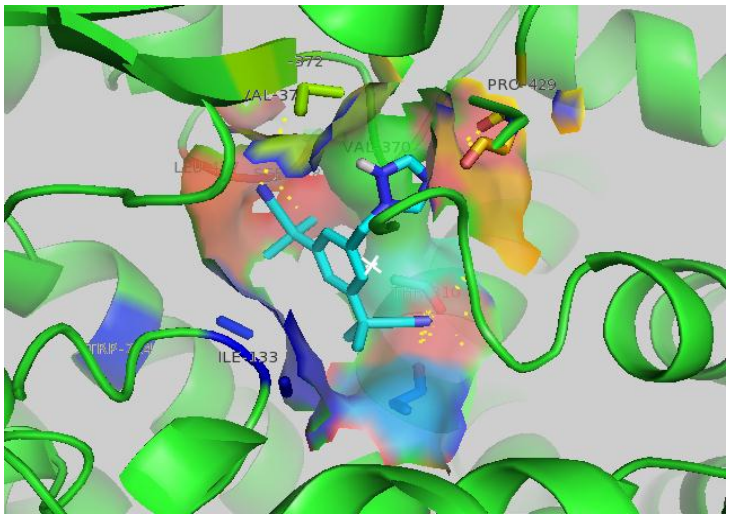

Figure 5: Binding pose of aromatase with anastrozole showing amino acid residue within 4 A

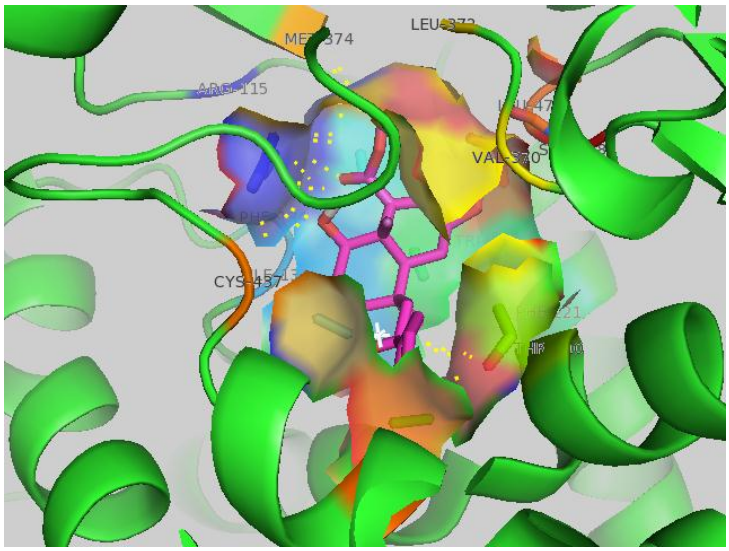

Figure 6: Binding pose of aromatase with borapetol showing amino acid residue within 4 A

DISCUSSION

Aromatase, a cytochrome P450 enzyme complex present in breast tissues, plays a significant role in the biosynthesis of important endogenous estrogens from androgens[16]. The source of estrogen production in breast cancer tissues is intra-tumoral aromatase, and inhibition of aromatase inhibit the growth stimulation effect of estrogens in breast cancer tissues. Consequently, aromatase is considered a useful therapeutic target in the treatment and prevention of estrogen-dependent breast cancer[17]. Compounds that are capable of inhibiting aromatase activity would likely offer a new way for protecting against breast cancer and its complications.

The Protein-Ligand interaction plays a meaningful role in structural based designing. In the present research, we have taken the receptor human aromatase and identified a novel inhibitor of this enzyme. The receptor (aromatase) was docked with anastrozole, a known drug that inhibit aromatase with the binding energy value of (-7.9kcal/mol) using Autodock/Vina (Figure 3, Table 1). Borapetol was docked against the same receptor and a binding energy of -9.4 kcal/mol was obtained (Figure 4, Table 2) making it a good ligand than anastrozole.

The present study helps us to comprehend the interaction between the ligand (borapetol) and receptor protein (aromatase) and also inquire their binding mode. Both anastrozole and borapetol were docked at the active site of aromatase. The amino acid residues in the active site are the main contributors to the aromatase-ligand interactions. The amino acid residues within 4A that stabilized the anastrozole -aromatase interaction are MET 374, PHE 430, SER 479, PRO 429, LEU 477, ARG 115, SER 478, ILE 133, TRP 224, PRO 429 (Figure 5), while those that stabilized borapetol-aromatase interaction are MET 134, MET 374, VAL 310, CYS 437, ARG 115, PHE 134, SER 478, THR 310, LEU 372, ALA 443, TRP 224 and PHE 221 (Figure 6)

The amino acid interacting with borapetol within 4A of ligand binding site but not found in the anastrozole are MET 134, VAL 310, CYS 437, PHE 134, THR 310, LEU 312, ALA 443 and PHE 221 These amino acids could be instrumental to the better interaction and the higher affinity that borapetol has for aromatase than anastrozole. From this research, we conclude that borapetol is a better aromatase inhibitor than anastrozole

CONCLUSION

The molecular docking studies with anastrozole and borapetol into the binding cavity of aromatase showed that borapetol has more binding affinity than anastrozole with better docking score. The results of this current study can be useful for the design and development of new aromatase inhibitor that can consequently be use to cure/manage breast cancer. Further studies using *in vitro* and *in vivo* experiments is encouraged.

REFERENCES

1. Adebayo A, Tan N, Akindahunsi A, Zeng G and Zhang Y. Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L.(Asteraceae). Pharmacognosy magazine. 2010; 6: 62.
2. Adebamowo CA and Ajayi OO. Breast cancer in Nigeria. West African journal Medicine. 2000; 19: 179-194.
3. Russo IH and Russo J. Role of hormones in mammary cancer initiation and progression. J Mammary Gland. Biol Neoplasia. 1998; 3: 49–61.
4. Kagawa N. Efficient expression of human aromatase (CYP19) in E. coli. Methods Mol Biol 2011; 705: 109-22.
5. Simpson ER and Davis SR. Minireview: aromatase and the regulation of estrogen biosynthesis--some new perspectives. Endocrinology. 2001; 142: 4589-94.
6. Simpson ER, Rubin G, Clyne C, Robertson K, O'Donnell L, Jones M and Davis S. Trends Endocrinol Metab. 2000; 11: 184.
7. Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M and Maloche C. Journal Clin Endocrinol Metab. 1996; 81: 1460.
8. Balunas MJ, Su B, Brueggemeier RW and Kinghorn AD. Natural Products as Aromatase Inhibitors. Anticancer Agents Med Chem. 2008; 8: 646–682.
9. Nabholtz, JM, Buzdar A, Pollak M, Harwin W, Burton G, Mangalik A, Steinberg M, Webster A and Von Euler M. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Journal of Clinical Oncology. 2000; 18: 3758-3767.
10. Lokman FE, Gu HF, Wan Mohamud WN, Yusoff MM, Chia KL and Östenson

CG. Antidiabetic effect of oral borapetol B compound, isolated from the plant *Tinospora crispa,* by Stimulating Insulin Release. Evidence-Based Complementary and Alternative Medicine. 2013; Article ID 727602.
11. Chou KC. Structural bioinformatics and its impact to biomedical science. Current Med Chem. 2004; 11: 2105–2134.
12. Wang SC, Ho IK, Tsou HH, Tian JN, Hsiao CF, Chen CH, Tan HK, Lin L, Wu CS et al. CYP2B6 Polymorphisms Influence the Plasma Concentration and Clearance of the Methadone S-Enantiomer. J Clin Psychopharmacol. 2011; 31(4): 463-469.
13. Chou KC, Wei DQ and Zhong WZ. Binding mechanism of coronavirus main proteinase with ligands and its implication to drug design against SARS. Biochem Biophys Res Comm 2003; 308: 148-151.
14. Sirois. J, Sayasith K, Brown KA, Stock AE, Bouchard N and Dore M. Cyclooxygenase-2 and its role in ovulation.*US* National library of medicine. 2011; 10: 373-85.
15. Gavanji S, Larki B and Mortazaeinezhad F. Bioinformatic prediction of interaction between flavonoids of propolis of honey bee and envelope glycoprotein GP120. International Journal of Scientific Research in Environmental Sciences. 2014; 2: 85-93.
16. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR and Bulun SE. Endocr. Rev. 1994; 15: 342.
17. Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, Graham- Lorence S, Sun T, Fisher CR, Qin K and Mendelson CR. Recent Prog Horm Res. 1997; 52: 185.