THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Kolaviron Inhibition of Aromatase of Breast Cancer, a Step-ahead of Exemestane, Letrozole, and Anastrozole: A Molecular Docking Studies

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Abstract:
Aromatase is a cytochrome P450 enzyme that synthesizes estrogen from androgen. Estrogen causes the proliferation of cancerous cells. However, inhibition of aromatase has proven to be helpful in the treatment of breast cancer in post-menopausal woman. Aromatase inhibitors (exemestane, letrozole and anastrozole) that are presently being used as first-line treatment of breast cancer have been reported to have side effects. In view of these, we have identified kolaviron a natural anti-aromatase biflavonoid from garcinia kola through molecular docking studies with the help of autodock software, to have the best interaction with amino residues of aromatase (-10.5Kcal/mol) on comparison with the third-generation aromatase inhibitors (exemestane -10.3Kcal/mol, letrozole -8.5 Kcal/mol and anastrozole – 8.2 Kcal/mol). Hence, kolaviron may be a novel and potent compound for the treatment of estrogen receptor positive breast cancer. However, there is need for further confirmation through wet experiment.

Keywords: *Cancer, Bioinformatics, molecular docking, exemestane, letrozole, anastrozole, kolaviron.*

1. Introduction

Breast cancer remain the most diagnosed cancer among female, over 182,000 women are diagnosed with breast cancer yearly in the United States (Jemal *et al*., 2011). Over half of a million death results yearly from breast cancer (Mouridsen *et al*. 2003). An estimated 80% of primary breast cancers are hormone sensitive because they contain estrogen receptor (ER) and/or progesterone receptor-positive cells (Keen & Davidson 2003, Nadji *et al*., 2005).

Aromatase is a member of the cytochrome P450 (CYP) super family enzyme and the product of the *CYP19* gene; it converts androstenedione to estrone and testosterone to estradiol (E2) (Dutta & Pant 2008) in peripheral tissues. Stimulation of the estrogen receptor by estrogen enhances the growth and development of breast cancer. Blocking of the estrogenic signaling pathway by antagonist has been the main focus of endocrine therapy. Tamoxifen, the first targeted cancer therapy against the estrogenic pathway has serious side effects ranging from increased risk of endometrial cancer and thromboembolism (Fisher *et al*., 1994, Jordan, 1995). The third-generation aromatase inhibitors (AIs), anastrozole, exemestane and letrozole are the current replacement for tamoxifen as first-line therapy in postmenopausal women with estrogen receptor positive breast cancer, due to their improved clinical efficacy (Nabholtz *et al.,* 2000; Bonneterre *et al.,* 2001) and lower toxicity.

The inhibition of aromatase enzyme by the third-generation aromatase inhibitors (anastrozole, letrozole, and exemestane) is the strategy for blocking the growth-inducing effects of estrogen in breast cancer, thereby leading to a drastic reduction in the level of estrogen in circulation. (Nabholtz *et al.,* 2000; Bonneterre *et al.,* 2001). However, there are concern over the risk effects of the third-generation aromatase inhibitors, estrogens play significant roles in the maintenance of normal bone turnover and normal bone mass, hence deprivation of estrogen for a long time is believed to cause osteoporosis and leads to bone fracture (Stefano Gonnelli and Roberto Petrioli., 2008). Aromatase inhibitors have been confirmed to cause Arthralgia within two months of onset of treatment (Burstein, 2007), also there have been reported cases of hot flashes with the use of the third-generation inhibitors. With the cases of the side effects associated with the third-generation inhibitors, there is need for a more potent and novel natural compounds with lower toxicities and greater inhibitory potential. Studies have shown that plant–derived bioactive compound inhibit aromatase (Campbell *et al*., 1993). Kolaviron, a biflavonoid complex from *Garciania kola* possesses chemopreventive ability (Farombi *et al*., 2005). With the aid of bioinformatics tools we have discovered that kolaviron is a potent aromatase inhibitor, the result of our molecular docking scores revealed that kolaviron gave the best interaction with our modeled aromatase enzyme than all the third-generation aromatase inhibitors.

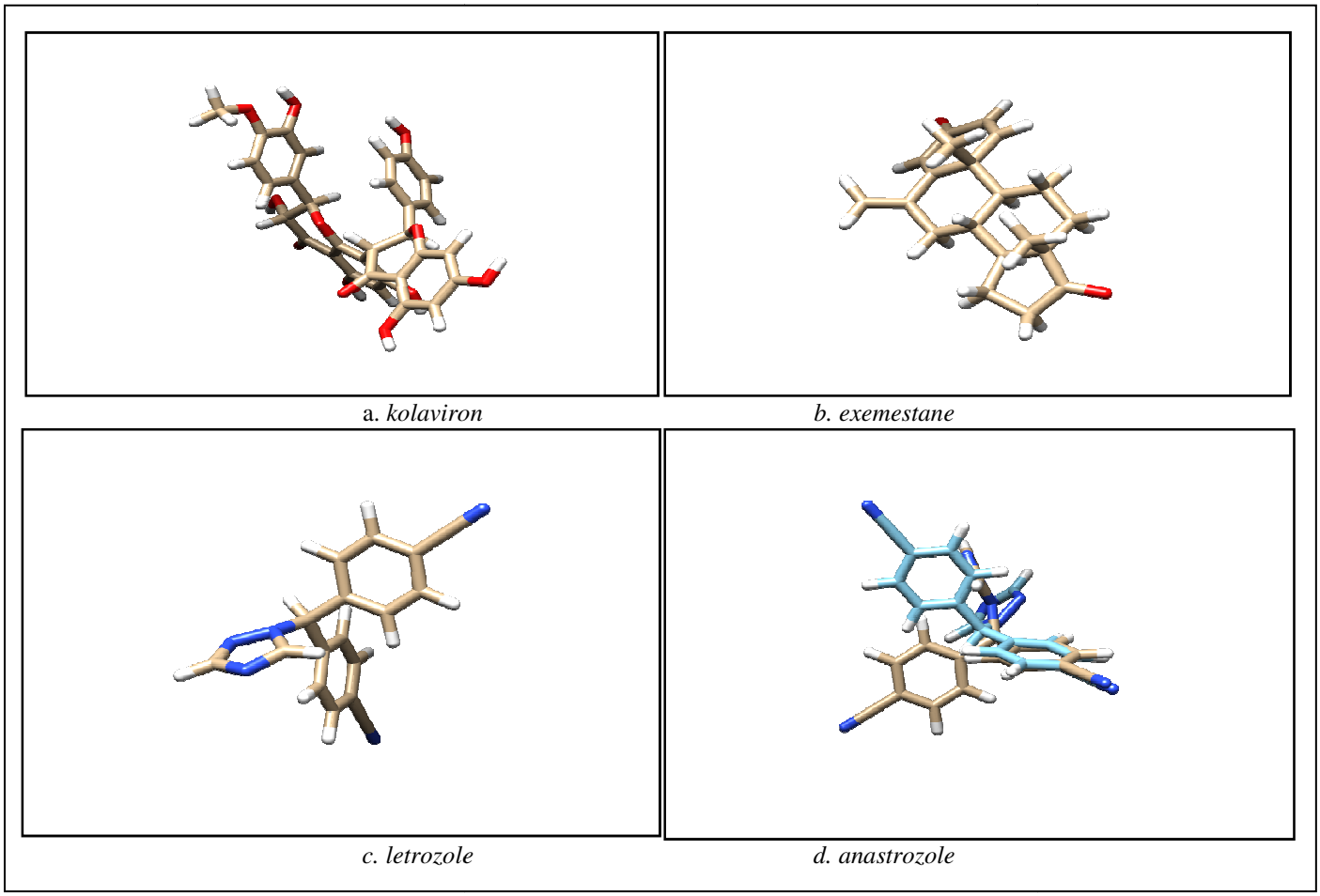

Figure 1: 3D Structures of Kolaviron, exemestane, letrozole, and anastrozole

2. Materials and Methods

2.1. Selection and Preparation of Macromolecule

Crystal structure of human placental aromatase complexed with breast cancer drug exemestane domain (Ghosh, 2012) (PDB ID: 3S7S) was retrieved from the protein data bank ((http: //www.rcsb.org). Human aromatase (Accession: NP_ 1125031_X GI: 281307079) "Fasta" file was downloaded from www.pubmed.org and was used in modeling the starting structure of aromatase used in the current study. The homology modeling was carried out in the Swiss Model Server (http://swissmodel.expasy.org). The co-ordinate file of template from protein data bank (PDB ID: 3S7S) was used to model the 3D structure of aromatase. Water and ligand coordinates were deleted prior to the molecular docking.

2.2. Selection and Preparation of Kolaviron, Anastrozole, Letrozole and Exemestane

The three-dimensional (3D) structures of kolaviron, anastrozole, letrozole and exemestane were collected from the pubchem database (Sayers *et al*., 2011). The optimized ligand molecules (kolaviron, anastrozole, lestrozole and exemestane) were docked into refined aromatase model using "LigandFit" in the AutoDock 4.2.

2.3. Molecular Docking

The docking of the of kolaviron, anastrozole, letrozole and exemestane to the binding site of aromatase was done using the autodock vina 4.2 [(Morris., *et al* 2009). Comparison of the results from docking protocols entails the removal of water molecules and ligand (exemetane) for better docking scores. The protein was treated as a rigid body (Chitranshi *et al*., 2013)**,** while the rotatable bonds of the ligands were set to be free**.** The grid box size was set at 86.08, 54.28 and 46.18 A° (x, y, and z) to include all the amino acid residues. The spacing between grid points was 0.375 angstroms.

3. Results

Compound Name	Binding Energy (Kcal/mol)
Exemestane	-10.3
Letrozole	-8.5
Anatrozole	-8.2
Kolaviron	-10.5

Table 1: Showing exemestane, letrozole, anastrozole and kolaviron with corresponding binding energies obtained from docking with aromatase using AutoDock program.

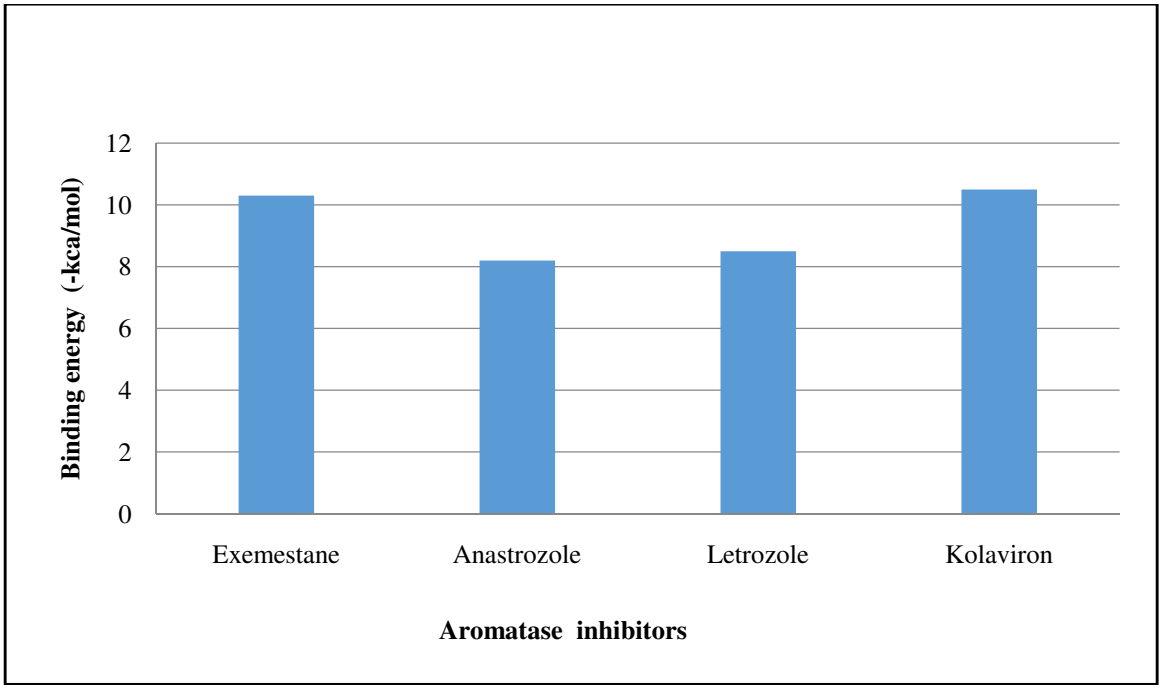

Figure 2: A bar chart showing the binding energy/ affinity of Exemestane, Anasrozole, Letrozole and kolaviron

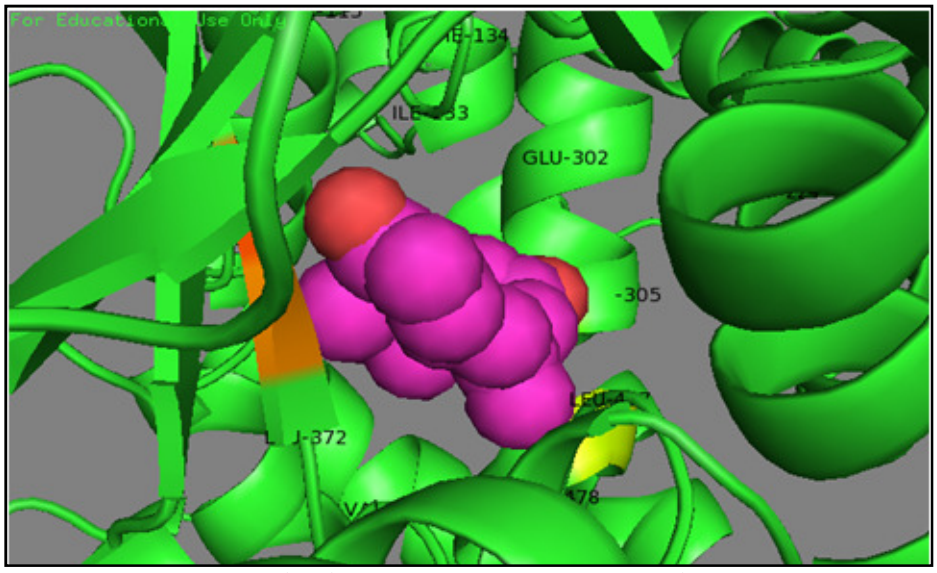

Figure 3: Molecular interaction of exemestane with aromatase

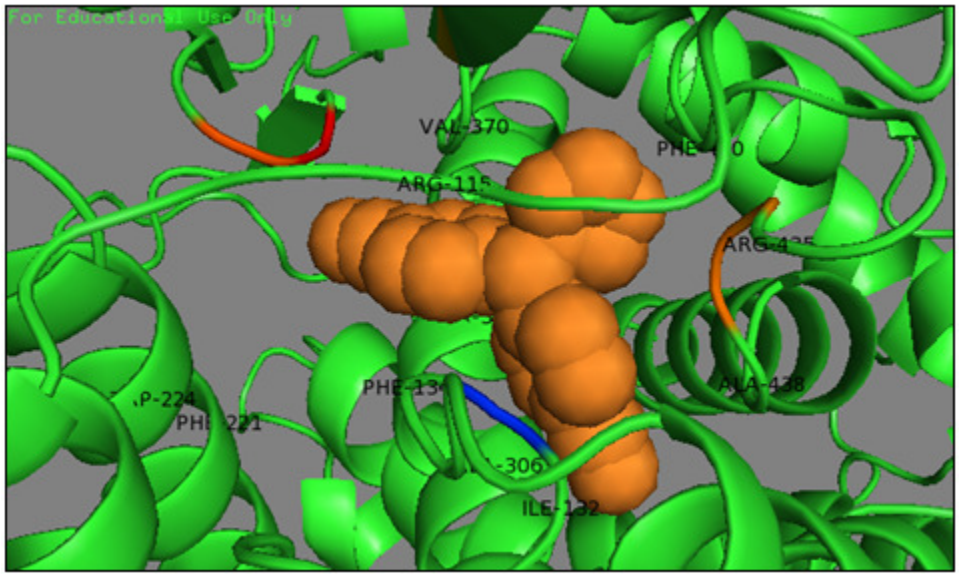

Figure 4: Molecular interaction of letrozole with aromatase

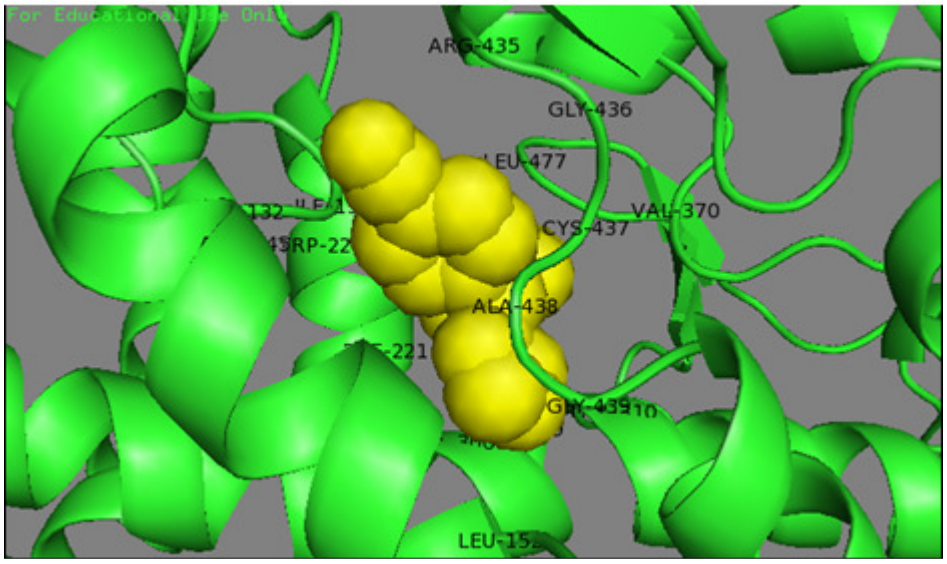

Figure 5: Molecular interaction of anastrozole with aromatase

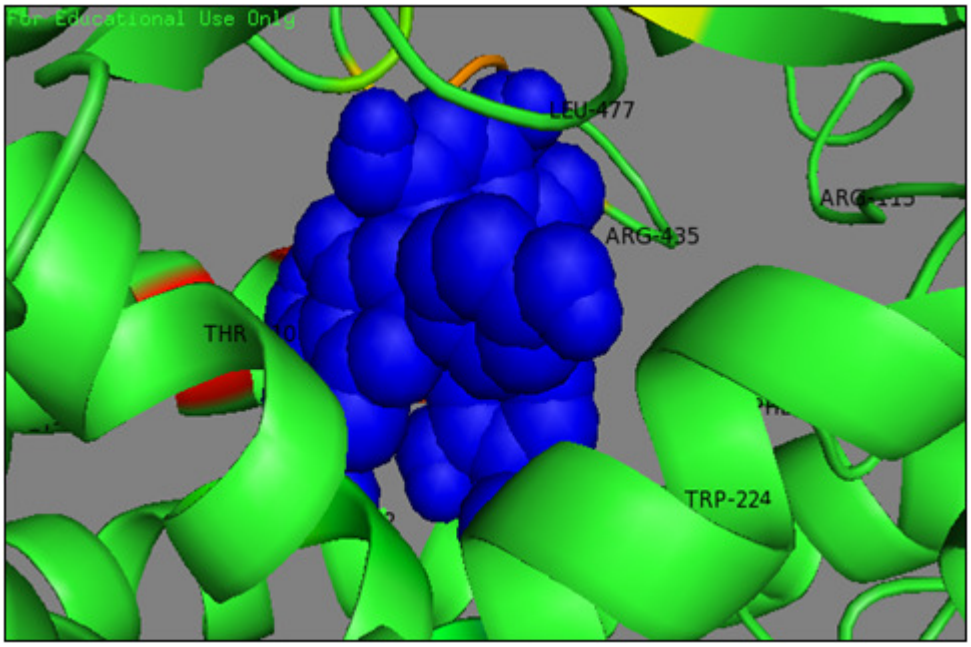

Figure 6: Molecular interaction of kolaviron with aromatase

4. Discussion

Aromatase belong to the cytochrome P450 (CYP) family enzymes, it converts androstenedione to estrone and testosterone to estradiol (E2) (Dutta & Pant 2008). Aromatase inhibitors block the activity of aromatase, thereby, preventing the synthesis of estrogen, which stimulates the growth and development of breast cancer.

The Protein-Ligand interaction plays a vital role in structural based drug design. In this present study, we have identified kolaviron from garcinia kola, by means of molecular docking studies to be a novel and likely more potent inhibitor of aromatase than any known third-generation aromatase inhibitors (exemestane, letrozole and anastrozole). Aromatase being the receptor was docked with exemestane, letrozole and anastrozole, these are established drugs that are administered in postmenopausal breast cancer patients, they inhibit aromatase with the binding energy value of (-10.3kcal/mol, -8.2kcal/mol and -8.5 kcal/mol) respectively using Autodock/Vina (Figure 3,4,5, Table 1). Docking of kolaviron against the same receptor (aromatase) gave us a binding energy of -10.5 kcal/mol (Figure 6, Table 1), this result indicates that kolaviron, is a better ligand than any of the third-generation aromatase inhibitors.

In this study, we have been able to understand the interaction between kolaviron and our receptor protein (aromatase) and also their binding mode were carefully elucidated. All the third-generation aromatase inhibitors drugs (exemestane, letrozole and anastrozole) and kolaviron were docked at the active site of aromatase by using the same grid center coordinate. The aromatase-ligand interactions are a function of the amino acid residues in the active site. The amino acid residues within 5A that stabilized the exemestane - aromatase interaction are MET 374, GLU 302, VAL 373, LEU 372, SER 478, VAL 370, LEU 477, THR 310, ASP 309, ALA 306, ILE 133, ARG 115, PHE 221, TRP 224, PHE 134 (Figure 3), letrozole-aromatase amino acid residue within 5A are 437, PHE 430, VAL 370, SER 480, LEU 477, VAL 373, VAL 370, SER 478, LEU 477, ARG 115, PHE 134, ILE 133, ILE 132, PHE 148, TRP 224, PHE 221 (Figure 4) anastrozole-aromatase amino acids residue within 5A are ALA 306, ALA 438, GLY 436, ARG 145, TRP 141, ARG 115, ILE 132, TRP 141, PHE 221, TRP 224, (Figure 5), while kolaviron-aromatase amino acids residue within 5A are GLU 302, TRP 224, ILE 132, ILE 133, ARG 115, ARG 435, GLY 436, VAL 373, VAL 370, PRO 429, ALA 443, TRP 224, PHE 148, THR 310, ALA 438, LEU 152 (Figure 6). In this present study, the result of our molecular docking scores revealed that kolaviron gave the best interaction with the amino acid residues, this may be as result of the hydrogen bonds interaction with GLU 302, THR 310 residues and pi–sigma stacking interaction between TRP 224 and PHE 148 of aromatase, exemestane-aromatase with close binding affinity also possess hydrogen bond interaction with SER 478, THR 310, ASP 309 and pi-sigma interaction at PHE 134, PHE 221, TRP 224 of aromatase. However, hydrogen bond interaction of GLU 302 is conserved in both kolaviron-aromatase and exemestane-aromatase. Anastrozole-aromatase showed the lowest amino acid residue interaction with the modelled protein (aromatase).

5. Conclusion

Based on the molecular docking scores of the present study, kolaviron is a better aromatase inhibitor than exemestane, letrozole and anastrozole. In addition, the different activities of kolaviron in the body should be examined, *in vivo* and *in vitro* assays need to be carried out to demonstrate its potential to hinder estrogen– mediated cell proliferations. Kolaviron as a chemopreventive agent should be consumed as a dietary supplement.

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