

## COMPUTATIONAL BIOLOGICAL STUDY OF AROMATASE INHIBITORS DOCKING WITH HUMAN PLACENTAL AROMATASE CYTOCHROME P450

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### ABSTARCT

**Objective:** In this current study analysis is done on the efficiency of the third generation drugs in comparison with tamoxifen, the first line drug used in the treatment of breast cancer in postmenopausal patients using computational approach. The drugs are docked with human placental aromatase cytochrome P450.

**Methods:** The tools and software used are Protein Data Bank, to retrieve the structure of the protein; pubchem compound database, to retrieve the chemical structure of the aromatase inhibitors; pharmacophore analysis and the docking analysis using Discovery Studio2.0.

**Results:** The results show that all the drugs are more favorable to bind with arginine (Arg) amino acid present in the active site of the protein. Letrozole shows good binding affinity with the active site of the protein.

**Conclusion:** Among the four drugs, letrozole shows more dock score of 76.566kcal/mol with internal energy of-9.759. On the other hand, tamoxifen shows least dock score of 48.494 kcal/mol having internal energy of-4.987. Thus, the docking study reveals that aromatase inhibitors drugs are more effective than tamoxifen for treating postmenopausal in women.

**Keywords:** Breast cancer, Aromatase inhibitors, Tamoxifen, Arginine (Arg), Letrozole

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### INTRODUCTION

An orderly sequence of events leading to the colonization of distal organs by malignant cells is often referred to as cancer metastasis [1]. Though the devastating consequences of metastasis have been recognized, the treatment of cancer is not that effective [2]. Breast cancer in 2013 has seen an estimate of 232,340 new cases of invasive breast cancer diagnosed among women, as well as an estimated 64,640 additional cases of in situ breast cancer. This cancer occurs much in women than men, and day by day the patients count on breast cancer is increasing rapidly [3]. Breast cancer research is going on throughout the world to find the remedy for treating the patients; the drug targets has also increased as also an increase in the specific pathway to inhibit the progress of cell growth[4]. We have also worked on the biological importance of flavonoids and phytochemicals [5-7] on HIV [8], diabetics and colon cancer [7].

This study is precisely on aromatase inhibitors as these inhibitors play a central role in the treatment of breast cancer [9]. Estrogen is important and promotes the growth and survival of normal and cancer epithelial cells. The chemical compound estrogen binds to the estrogen receptor (ER) and the activation of the cell progression takes place. The active estrogen receptor tends to bind with promoter gene present in the nucleus, which regulates the gene activity and translates the protein [4, 9]. The activated receptor in turn binds to gene promoters in the nucleus and activates many other genes [9, 10]. The activated gene products are thereby responsible for cell division, inhibition of cell death, new blood vessels formation and protease activity. Rapid expression of estrogen receptor is found at earlier stages of breast cancer. Nearly 70% cancer mainly depends on the over-expression of estrogen receptor [11, 12].

Aromatase inhibitor inactivates the production of estrogen from androgens, by suppressing aromatase enzyme activity. The breast cancer patients treated with aromatase inhibitors show, low level of estrogen secretion in the tumor cells [13]. The aromatase enzyme is responsible for the conversion of androstenedione to estrone and testosterone to estradiol, any mutation or overexpression of estrogen can be controlled only by inhibiting the conversion process.

Hence, aromatase is chosen as important drug target protein in breast cancer treatment [14, 15]. In this current study, we have tried to generate the individual pharmacophore and common feature pharmacophore of aromatase inhibitors and tamoxifen. The most widely used nonsteroidal antiestrogen in the treatment of breast cancer in all its stages is tamoxifen [16]. The main anti-estrogen treatments in use consist of third generation aromatase inhibitors, such as exemestane, letrozole, and anastrozole [17].

In the present work, the potential of the aromatase inhibitors exemestane, letrozole and anastrozole in the treatment of breast cancer has been analyzed using computational approach. The pharmacophore features gives an idea about chemical properties and biological activity of the drugs. The docking of aromatase inhibitors and tamoxifen with human placental aromatase cytochrome P450 reveals the role of amino acid, not only at the active site but also at the molecular or atomic level interaction of these compounds. The secondary structure of the drug target protein is shown in fig. 1.

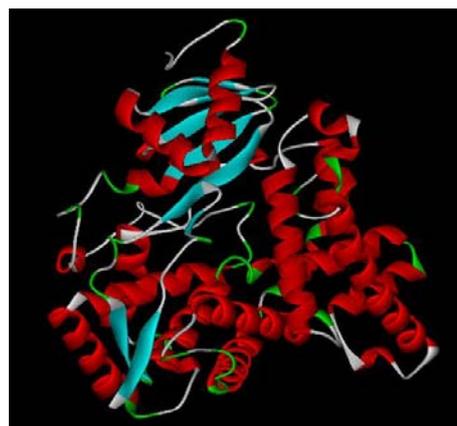


Fig. 1: Secondary structure of the target protein

## MATERIALS AND METHODS

### Retrieval of protein from protein database

The structure of the drug target protein, the human placental aromatase cytochrome P450 and its X-ray crystallographic structure with 2.90 Å resolution was retrieved from protein data bank with its Identification number as 3EQM, commonly known as PDB ID, is a complex with androstenedione.

### Protein preparation

The raw protein is retrieved from protein data bank and all other chemical moieties are present in protein are removed. Energy

minimization was performed to remove the bad steric clashes with 1000 steps at RMS gradient of 0.1 and 0.03 respectively. Priorly a suitable force field CHARMM, available with Accelrys life science software [18] was applied.

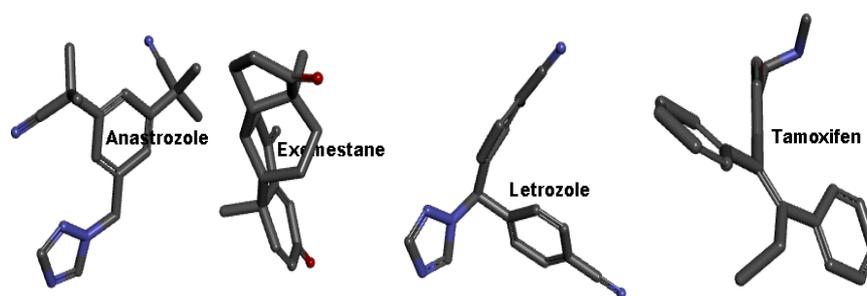
### Chemical structure retrieval

The aromatase inhibitors were obtained after literature analysis and its respective chemical structure is retrieved from the PubChem compound database (<https://pubchem.ncbi.nlm.nih.gov/>).

The list of compounds with their corresponding ID is shown in the table 1 and their structure in fig. 2.

**Table 1: List of compounds retrieved from pubchem database**

Drugs	Pubchem ID	SMILES notation
Anastrozole	CID: 2187	<chem>CC(C)(C#N)C1=CC(=CC(=C1)CN2C=NC=N2)C(C)(C)C#N</chem>
Exemestane	CID: 60198	<chem>CC12CCC3C(C1CCC2=O)CC(=C)C4=CC(=O)C=CC34C</chem>
Letrozole	CID: 3902	<chem>C1=CC(=CC=C1C#N)C(C2=CC=C(C=C2)C#N)N3C=NC=N3</chem>
Tamoxifen	CID: 5376	<chem>CCC(=C(C1=CC=CC=C1)C2=CC=C(C=C2)OCCN(C)C)C3=CC=CC=C3</chem>



**Fig. 2: The structure aromatase inhibitors drugs**

### Qualitative pharmacophore analysis

The individual structure of each compound is retrieved and qualitative pharmacophore analysis task was performed by optioning best conformation generation with maximum generations of 255 conformations per compound. Pharmacophores are aligned based on the cat-hip hop algorithm. In this study, we have tried to find the individual pharmacophore of each structure.

### Molecular docking

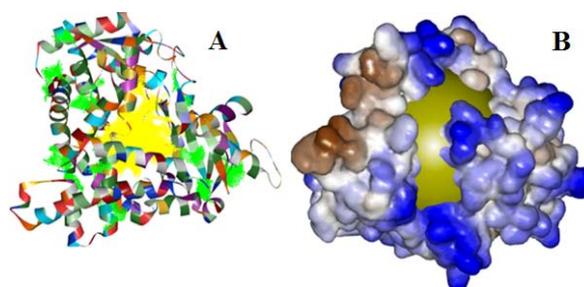
Structure-based drug design is the method of performing the docking using the known protein docking with known ligands. This is the frequently used method of analyzing the receptor-ligand interaction between the active site of the protein and the chemical molecules. In this current study, the PDB ID 3EQM, structure of human placental aromatase cytochrome P450 is docked with the drugs anastrozole, exemestane, letrozole and tamoxifen.

## RESULTS AND DISCUSSION

### Cavity prediction of protein

*In silico* binding site prediction is one of the important tasks in bioinformatics to carry to docking studies. Leis *et al.*, 2010 describes that prediction of the accurate binding site on the protein surface plays an important role for rational based drug design process with relevance to medical applications. He states that many cavities or binding site prediction tools are developed rapidly to find the exact surface of the protein-binding site [19]. In this study, the binding site of the protein is predicted based on erase and flood filling algorithm. There are totally 14 sites has been predicted on the by flood filling algorithm available in accelrys discovery studio 2.0v software in a tool for binding site prediction. Fig. 3 shows the binding site of the drug target protein. In the fig. 3: A yellow color shows largest binding site whereas other binding sites is shown in green color. Fig.

3: B shows hydrophobic surface of the protein with yellow color in the middle as binding site of the protein.



**Fig. 3: Binding site of the protein**

### Chemical feature of the drugs

The pharmacophore concept describes the chemical and functional features of the ligand and its importance for biological processes. Dhivya *et al.*, 2012 [20] describes the importance of pharmacophore-based screening of natural compounds for colon cancer and another study on same year shows the importance of plant metabolite axillirain and its pharmacophore analogy virtual screening of compounds for HIV based on structure-based drug designing study for targeting the drug target protein HIV-1 protease[7]. The functional group of the each drug plays a vital role in biological activity. Replacement or substitution of a functional group meant for a specific biological activity, by another functional group may increase or decrease the activity of the drug or drug candidate.

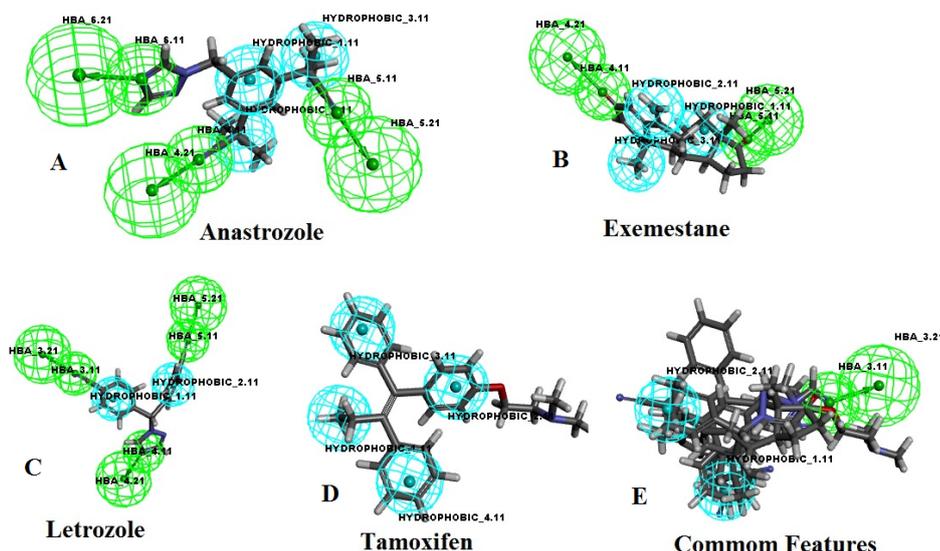


Fig. 4: Pharmacophore of the drugs

Fig. 4 shows the individual pharmacophore features of the drugs. Commonly green color indicates hydrogen bond acceptor (HBA) and cyan color indicates hydrophobic region. 4A: shows the six pharmacophore feature of the drug anastrozole, among them three are HBA and another three features are hydrophobic. In fig. 4B: exemestane shows five different features, where three of them are hydrophobic and two are acceptors. Similarly, fig. 4C & D shows letrozole with five pharmacophore features with three acceptors, two hydrophobic, and tamoxifen with four hydrophobic features respectively. Whereas, the common features of the four drugs show two hydrophobic features and one acceptor.

This analysis shows that individually each drug has more pharmacophore features than the common features generation using cat-hip hop. The advantage of common feature generation is to fit all the drugs to the aligned pharmacophore of each drug in different conformations. In some cases, the drug which does not fit to that 3-D spatial arrangement of pharmacophore are neglected from the group. Hence it is necessary to generate the individual and common feature pharmacophore of drugs or drug candidates. The table 2 shows the fit value and different conformation generated for aligning common feature pharmacophore generation for the four drugs. A fit value reveals the aligning of the compound to common pharmacophore feature generated. Among the four drugs anastrozole shows more fit value and letrozole shows least fit value.

Table 2: Common feature generation of drugs

Drugs	Fit value	Confirmation number
Anastrozole	3	21
Tamoxifen	2.70735	67
Exemestane	2.27961	48
Letrozole	1.32401	91

Both the drugs and drug target protein is known, and its binding site is created automatically using the flood-filling algorithm. It is a continuous process of docking the drugs to the different sites of the drug target protein until the best ten poses created for drugs to the binding site of the protein is over. Ligand fit algorithm is used for docking at different sites. Initially the study begins at site 1 to find the interaction, least energy value with good dock score is considered as efficient docking. In case if, there is no pose or no more interaction the study extends for other sites to check the possibility of the drug binding to the site. The site 1 with three partitions due to large volume 879.125 Å with a point count of 7033 in equal grid spacing of 0.5 (X), 0.5 (Y), 0.5 (Z) direction respectively. The drugs were docked with a sphere site being defined as 82.18 (X), 50.817 (Y), -49.482 (Z). Fig. 6 shows the receptor-ligand interaction of the drug with the amino acid present at the binding site of drug target protein and its dock score, whereas fig. 5 shows the dock score and internal energy of the drugs at site 1.

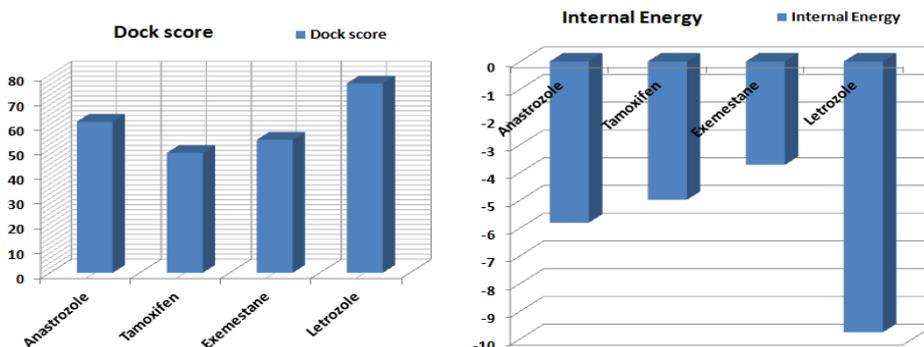


Fig. 5: Docking and internal energy of the drugs

On comparison with tamoxifen, exemestane is effective and well tolerated and offers significant early improvement in time to tumor progression in the treatment of postmenopausal treatment [21]. letrozole, anastrozole and exemestane are third generation

inhibitors where the former two are non-steroidal and the later one steroidal [22]. Lamb, H. M and Adkins, J. C recommends letrozole for postmenopausal treatment with advanced breast cancer whose disease has progressed on [23]. The results of our analysis also

suggests that letrozole, exemestane followed by anastrozole are more potent than tamoxifen, in the treatment of breast cancer. Regarding side effects, aromatase inhibitors are not found associated with enhanced risk of cardiovascular disease, and

enhanced bone loss is prevented by adding bisphosphonates in concert for those at danger of developing osteoporosis [25]. Letrozole [23], anastrozole [25] and Exemestane [26], are shown to be more potent than tamoxifen.

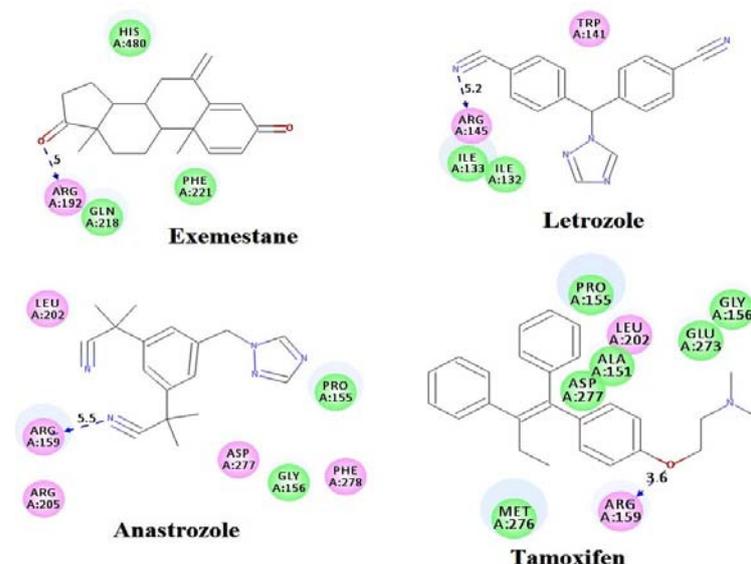


Fig. 6: Receptor-ligand interaction

## CONCLUSION

The potential of aromatic inhibitors in the treatment of breast cancer has been analysed by computational methods. The present study shows that aromatase inhibitors are much more effective than tamoxifen in postmenopausal women. Among four drugs, letrozole shows more dock score of 76.566kcal/mol with internal energy of -9.759. On the other hand, tamoxifen shows least dock score of 48.494 kcal/mol with internal energy of -4.987. Thus, the docking study reveals that aromatase inhibitor drugs are more effective than tamoxifen for postmenopausal treatment in women.

## CONFLICT OF INTERESTS

Declared none

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