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fungal pigments as potential aroma-  
tase inhibitors**

## Computational drug designing of fungal pigments as potential aromatase inhibitors

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### Abstract

The existing aromatase inhibitors produced unwelcome effects impose the discovery of novel drugs with privileged selectivity, a reduced amount of toxicity and humanizing potency. In this study, we illuminate the binding mode of polyketide azaphilone pigments monascin, ankaflavin, monascorubrin and monascorubramine isolated from *Monascus* fungus to the aromatase by molecular docking. The 3-dimensional structure of aromatase enzyme (PDB: 4KQ8) was obtained from the Protein Data Bank. PatchDock docking software was used to analyze structural complexes of the aromatase with monascus pigments. Comparatively, the AutoGrid model presented the most briskly constructive binding mode of monascin to aromatase. Docked energies in kcal/mol are: monascin:-13.2; monascorubramine:-12.8, monascorubrin:-12.3; ankaflavin:-10.5. These outcomes exposed these ligands could be potential drugs to treat hormone dependent breast cancer.

## Introduction

Monascus pigments biosynthesized from fungi of genus *Monascus*, typically comprised six major valuable bioactive polyketide azaphilone pigments; ankaflavin (yellow); rubropunctatin (orange), monascin, rubropunctamine (purple red), monascorubrin, and monascorubramine, (Lee et al., 2006; Mapari et al., 2010; Lee et al., 2012; Figure 1).

Breast cancer (BC) is one of the most widespread and multifarious disease that accounts in women approximately 22.9% of all cancers in world. The means and molecular biology of breast cancer has not been completely investigated (Standish et al., 2008) and limitations of existing therapies prompt investigators to carry on penetrating for more effective molecules (Sotiriou et al., 2006; Sotiriou and Pusztai, 2009).

Estrogen hormones are caught up in the growth of breast cancer which is most frequent in post-meno-

pausal women (Maiti et al., 2007). Aromatase is a cytochrome P450 enzyme playing a significant role in the translation of androgen to estrogen (Jongen et al., 2005). Aromatase inhibitors can stop the creation of estrogen, as a result can orderly reduces the development of breast cancer cells. Therefore, aromatase inhibitors have become attractive therapy (Chomcheon et al., 2009; Sureram et al., 2012).

Although various compounds have been studied for their aromatase inhibition potential but complexity of enzyme structure lemmatizes the use of these compounds. An alternative strategy to design new inhibitors relies on ligand-based virtual screening by using different compounds.

Therefore in this study, we have used natural polyketide pigments of *Monascus* fungus for virtual screening. Our study would be supportive in adding new lead molecules and drug targets to smooth the progress of the diagnosis and the management of breast cancers.



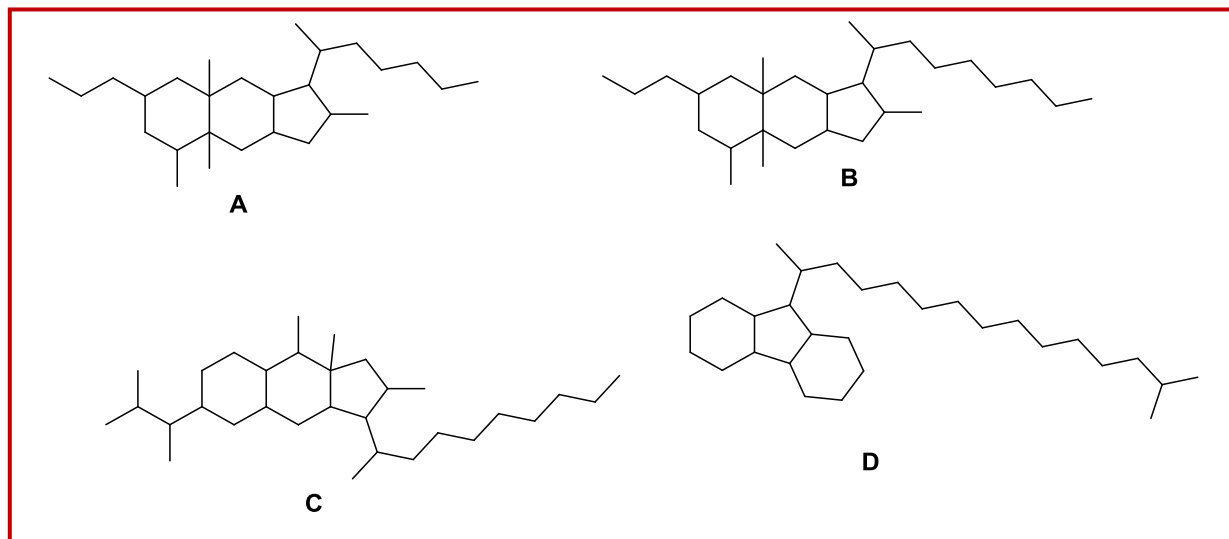


Figure 1: Different monascus pigments used as aromatase inhibitors (A) Monascin, (B) Ankaflavin, (C) Monascorubrin, (D) Monascorubramine

## Materials and Methods

**Accession of target protein:** The 3-dimensional structure of aromatase (cytochrome p-450) enzyme (PDB: 4KQ8) was downloaded from the RCSB protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>).

**Ligand selection:** The chemical structures of monascin, monascorubrin, monascorubramine and ankaflavin were obtained from PubChem database. structures were drawn by using ChemBioDraw and MOL2 format of these ligands were changed to PDB file using Open Babel tool earlier to upload onto PatchDock virtual software.

**Target and ligand optimization:** For docking analysis, PDB coordinates of the target protein and ligands molecules were optimized by Drug Discovery Studio version 3.0 software and UCSF Chimera tool respectively. These coordinates had minimum energy and stable conformation.

**Analysis of target active binding sites:** The active sites are the coordinates of the ligand in the original target protein grids and these active binding sites of target protein were investigated using the MetaPocket 2.0 virtual tools (Huang, 2009).

**Molecular docking analysis:** A computational approach, ligand-target docking was adopted to investigate the aromatase structural complexes (cytochrome p-450) as target with chosen compounds (ligands) in order to comprehend the structural basis of this particular protein target. To begin with, Platinum software web server was used for the investigation of protein-ligand attraction of complexes and to determine their hydrophilic or hydrophobic properties (Pyrkov et al., 2009). As a final point, these complexes were subjected to docking by using PatchDock virtual docking software

(Schneidman-Duhovny et al., 2005). The energy of interaction of ligands with the target enzyme is depicted as "grid point". At every step of the simulation, the ligand-protein interaction of energy was monitored by atomic affinity potentials computed on a grid. The remaining parameters were set as default.

## Results and Discussion

Molecular docking is an important source which depicts helpful inquiries regarding drug-receptor interactions and is repeatedly used to forecast the binding orientation of small molecule behaving as potential candidates for drug with their target protein to guess their potential activity and affinity towards their target (Vijesh et al., 2013). Therefore molecular docking studies of biologically active fungal pigments provided superior perceptive of the drug-receptor interaction.

The minimum binding energy indicated that the aromatase protein (target enzyme) was successfully docked with ligands molecules (Table I). The possible binding modes of ligands at aromatase active sites have been shown in Figure 2. Aromatase protein residues Arg, Met, Phe, Ser, Tyr, Pro, Asn, Glu, Val, Lys, Gln, Ala, Ile was formed H-bond with ligands molecules. Monascin showed relatively good binding affinity (-13.2 kcal/mol) as compared to other ligands.

The division of hydrophilic or hydrophobic properties of aromatase and its binding site anticipated against the surface of the ligands, and their complementarity was inquired. Less hydrophilicity of aromatic groups is in common practice. These stacking interactions are based to grade the molecular docking to investigate the drug-target interaction. Taking into account the hydrophobic properties, molecular hydrophobicity potential was

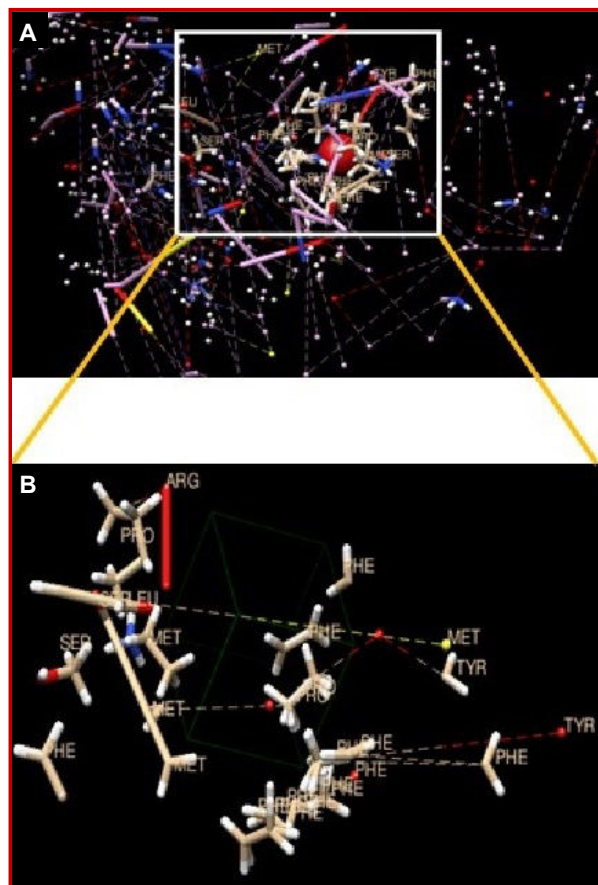


Figure 2: Potential binding sites of aromatase (cytochrome p-450) with binding site center X: 78.117, Y: 45.497, Z: 58.722 indicating amino acids residues: Arg, Met, Phe, Ser, Tyr, Pro, Asn, Glu, Val, Lys, Gln, Ala, Ile (A) Binding sites for ligands (B) maximized region

calculated for these interacting molecules (aromatase protein target and ligands) (Muhammad et al., 2014) which were the bases for the study of progress of molecular dynamics run base on hydrophobicity clustering on the surface of membrane also shows the membrane-molecule interaction.

The docking of ligands-aromatase target came up that all the computationally forecast lowest energy complexes of aromatase are stabilized by stacking interactions and intermolecular hydrogen bonding. It was also originated that A, SA, OA, HD, N are the ligand atoms are responsible for in docking with the enzyme. The AutoGrid model came up with the consequences that the most energetically acceptable binding mode of monascin to aromatase. The monascin as ligand was docked into the generated combined grids, the RMSD from native pose and the binding energy were investigated. It is found that the weight averaged grids performs excellently. Base on RMSD values it was found that the ligand showed significant interaction with target proteins as compared to other compounds. Beside RMSD clustering, PatchDock has deliberate the binding free energies of these interactive molecules to discover the finest binding mode. The intended final docked energies for monascin was -13.2 kcal/mol, for ankaflavin -10.5 kcal/mol (Figure 3), for monascorubrin -12.3 kcal/mol and -12.8 kcal/mol monascorubramine were observed (Figure 4). Docking results came up with the statement that these ligands molecules can interact with aromatase protein target more significantly and can help in the study of cancer research.

Table I				
Energy and RMSD values obtained during docking analysis of ligands molecules and aromatase enzyme as target protein				
SL. No.	Complex	Binding energy	RMSD/UB <sup>a</sup>	RMSD/LB <sup>a</sup>
1	Aromatase_Monascin	-13.2	0	0
2	Aromatase_Monascin	-12.8	1.1	1.5
3	Aromatase_Monascin	-12.3	2.2	1.8
4	Aromatase_Monascin	-11.2	2.9	1.9
1	Aromatase_Ankaflavin	-10.5	0	0
2	Aromatase_Ankaflavin	-9.4	2.3	1.3
3	Aromatase_Ankaflavin	-8.7	2.9	1.8
4	Aromatase_Ankaflavin	-8.1	3.7	2.0
1	Aromatase_Monascorubrin	-12.3	0	0
2	Aromatase_Monascorubrin	-11.4	2.2	1.6
3	Aromatase_Monascorubrin	-9.3	3.1	1.9
4	Aromatase_Monascorubrin	-7.9	3.9	2.1
1	Aromatase_Monascorubramine	-12.8	0	0
2	Aromatase_Monascorubramine	-12.1	2.5	1.9
3	Aromatase_Monascorubramine	-10.5	3.4	2.1
4	Aromatase_Monascorubramine	-9.9	4.1	3.0

<sup>a</sup>RMSD/UB: Root mean square deviation/upper bond; RMSD/LB: root mean square deviation/lower bond

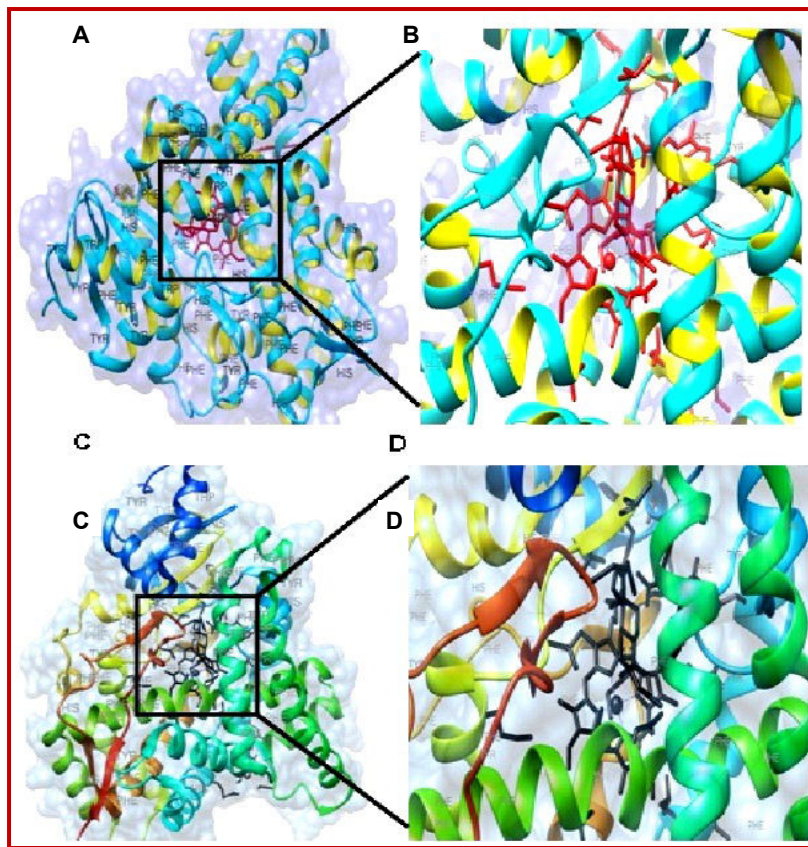


Figure 3: Visuals of docking procedure obtained using PatchDock virtual tool (A) The AutoGrid dimensions between monascin and aromatase (cytochrome p-450) are: grid center X: 19.1414, Y: 21.2513, Z: 11.3412 with dimension (Angstrom) X: Y: Z: 25.000 (B) maximized region presenting confirmation and pose of monascin ligand (C) The AutoGrid dimensions between ankaflavin and aromatase (cytochrome p-450) are: grid center X: 20.0211, Y: 23.2021, Z: 12.1598 with dimension (Angstrom) X: Y: Z: 25.000 (D) maximized region presenting confirmation and pose of ankaflavin ligand

## Conclusion

Docking studies of the monascin, ankaflavin, monascorubrin and monascorubramine with aromatase enzyme showed that these natural compounds are good ligands which dock well with aromatase target.

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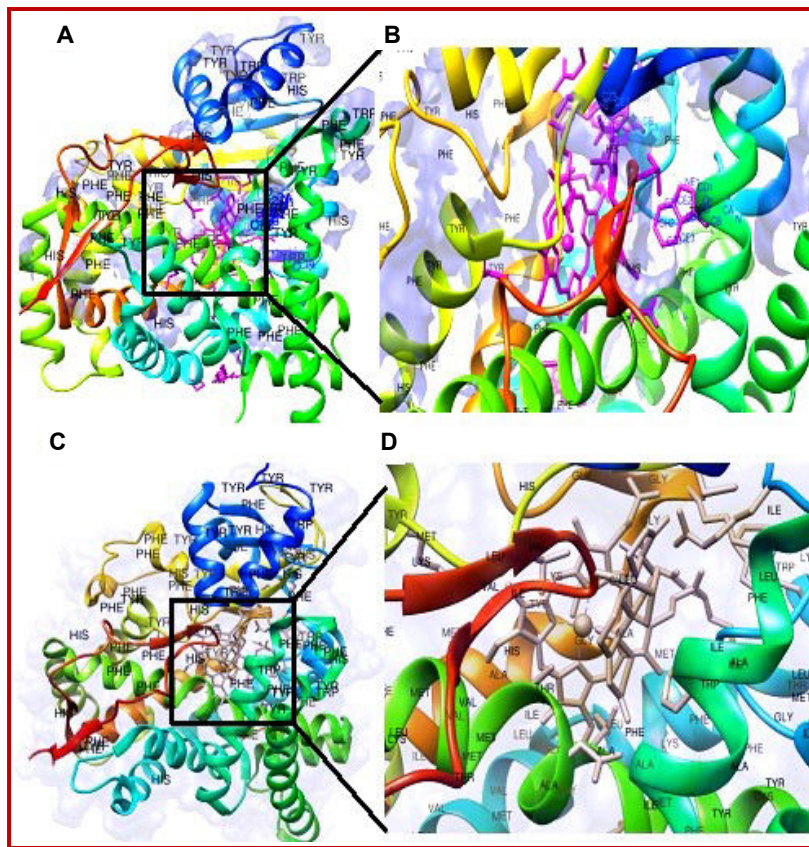


Figure 4: Visuals of docking procedure obtained using PatchDock virtual tool (A) The AutoGrid dimensions between Monascorubrin and aromatase (cytochrome p-450) are: grid center X: 18.1001, Y: 22.2412, Z: 10.1201 with dimension (Angstrom) X: Y: Z: 25.000 (B) maximized region presenting confirmation and pose of Monascorubrin ligand (C) The AutoGrid dimensions between Monascorubramine and aromatase (cytochrome p-450) are: grid center X: 19.0011, Y: 21.1231, Z: 11.0251 with dimension (Angstrom) X: Y: Z: 25.000 (D) maximized region presenting confirmation and pose of Monascorubramine ligand

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