DOCKING STUDIES OF NOVEL CHALCONES OF QUINOLINE SCAFFOLD AS INHIBITORS OF AROMATASE AND EPIDERMAL GROWTH FACTOR RECEPTOR FOR BREAST CANCER THERAPY

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Abstract

Breast cancer causes second largest cancer deaths among women globally. Aromatase enzyme and epidermal growth factor receptor (EGFR) act as an important targets for breast cancer therapy. The present study investigates the computational docking interaction of some new chalcones comprising benzimidazole and quinoline against these targets. The docking study of the ligands were carried out against aromatase (PDB ID 3S7S) and EGFR (PDB ID 3POZ) using Schrodinger suite. The dock score shows that the ligands have better binding interaction with the target aromatase than the standard exemestane. The ligand 10A and 15A showed good binding with aromatase whereas ligand 1A bind with EGFR as a better inhibitor. All the compound obeyed Lipinski's rule of five and can be considered as novel drug targets for breast cancer therapy.

Keywords: Docking, aromatase, epidermal growth factor receptor, targets

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1. INTRODUCTION

Breast cancer is the most prevalent type of cancer globally especially in women. As per the 2020 scenario 2.3 million cases due to breast cancer were reported in women with 685000 deaths[1]. Approximately half of breast cancers develop in women who have no identifiable breast cancer risk factor other than gender (female) and age (over 40 years). Hormone receptors-bearing tumours are correlated with the mortality rate of the majority of breast cancer patients. Estrogen receptors are the key signalling molecules in hormone-dependent breast cancer that control a variety of cell functions, either directly through transcription factors in the nucleus or indirectly through interacting with other receptors and their neighbouring pathways, such as EGFR/IGFR.[2] The promotion of cell proliferation and differentiation by the EGFR and IGFR signalling pathways has been thoroughly documented in the literature.

Estrogens are known to be important in the growth of breast cancers in both pre- and postmenopausal women. The enzyme Aromatase converts testosterone to estradiol, which is the final and rate-limiting step in estrogen biosynthesis. Aromatase belongs to a class of cytochrome p450 superfamily of enzymes.[4] It is a membrane bound protein which is localized in the endoplasmic reticulum. Using enzyme activity measurement, immunocyto chemistry, and RT-PCR analysis, it has been discovered that the expression of aromatase is higher in human breast cancer tissue than in healthy breast tissue. Studies employing transgenic mice, cell culture, and animal trials using breast cancer cells that have been aromatase-transfected have all shown that in situ generated oestrogen promotes breast tumours more effectively than circulating oestrogens. Due to their superior therapeutic impact over tamoxifen, an oestrogen modulator, aromatase inhibitors (AIs) are now used in clinics to treat estrogen-dependent breast tumours. Aromatase inhibitors (AIs) such as letrozole inhibit the enzymatic activity of aromatase and thereby diminish the estrogenic capability throughout the body. They are widely used as the first line therapy in postmenopausal women with ER+ breast cancer.[5,6]

Epidermal growth factor receptor (EGFR) is a member of the ErbB family, a family of tyrosine kinase receptors with growth-promoting effects. Overexpression or/and abnormal activation of ErbB family of receptor tyrosine kinases (mainly EGFR and/or HER2) has been associated with the proliferation of breast cancer cells and tumour progression. It has been reported that the expression of both EGFR and HER2 is inversely correlated with estrogen receptor (ER) status, and EGFRHER2 heterodimers have been shown to increase the metastatic potential of breast cancer cell lines. A variety of molecules have been developed as inhibitors of EGFR for treating breast cancer including Panitumumab and Cetuximab.

Chemically, chalcones or (E)-1,3-diphenyl-2-propene-1-one are α, β- unsaturated carbonyl compounds. They are biosynthetically useful moiety with wide variety of pharmacological actions and act as precursor for majority of flavanoids.[7] Several natural and (semi) synthetic chalcones have shown anti-cancer activity due to their inhibitory potential against various targets namely, aromatase and 17-β-hydroxysteroid dehydrogenase, 5α-reductase, topoisomerase-II, HDAC/Situin-1, cathepsin-K, proteasome, VEGF, VEGFR-2 kinase, JAK/STAT signalling pathways, and tubulin.[8] The present study focus on generating new chalcone molecules as target inhibitors for both aromatase and EGFR for providing new treatment profile in breast cancer. The successful application of docking and ADMET properties will result into discovering of novel and potential anticancer agents based on chalcone scaffold by inhibiting aromatase and EGFR enzyme in the treatment of breast cancer.

2. MATERIALS AND METHODS

2.1 Ligand generation

The structure of the proposed ligands for docking against aromatase and EGFR enzymes were drawn using chemdraw. Three-dimensional (3D) structures of all atoms in molecules can be generated using LigPrep. While preparing ligands for molecular docking two-dimensional (2D) structures are converted into 3D structures for generating variations, correction, verification, and optimization of the structures. Open babel software was used to convert the structures into mol2 format prior to docking. The energy minimisation of the structures was done using OPLS until the structures gained the lowest possible energy. LigPrep software was used to investigate all potential tautomers of ligands while keeping their stereochemistry, and it generated numerous conformations using confgen. Additionally, ligand ionisation states were produced using the Epik 3.4 programme.

2.2 Obtaining protein structure

The crystal structure of the target proteins were downloaded from PDB (protein data bank). The PDB id of aromatase is 3S7S and that of EGFR

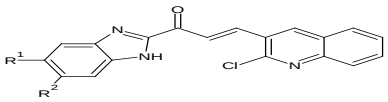
kinase is 3POZ.[9,10] Crystal structure of human placental aromatase enzyme was complexed with breast cancer drug exemestane. The R value of the crystal structure was 0.256 with a resolution of 3.21 Å. EGFR kinase domain was complexed with TAK 285 with a resolution of 1.50 Å and R value 0.243. The targeted protein 3S7S and 3POZ belong to homosapiens.

2.3 Protein preparation

The protein was prepared by using Protein Preparation Wizard of Schrodinger Suite. The downloaded proteins were energy minimised by adding hydrogen atoms and removing the water molecules and verifying the protonation state especially near to the binding sites. The proteins were then prepared for docking using the maestro version by adding the missing residues or loops and assigning atomic charges both in aromatase and EGFR kinase.

2.4 Docking studies

In order to include the cofactor and substrate binding sites, the receptor grid generation file—which was employed throughout the docking study—was defined as an enclosing box at the centroid of the co-crystallized ligand (3S7S) and (3POZ). The Glide's receptor grid generation wizard was used to generate a three-dimensional (3D) grid with a maximal size of 20 × 20 × 20 Å with 0.5 Å spacing. The receptor grid for 3S7S and 3POZ were generated by specifying the binding (active) site residues, which was identified by SiteMap tool. Once the receptor grid is generated, the ligands are docked to the protein using Glide. Finally, flexible docking using the extra-precision docking mode in the Glide docking module was completed. The best docked pose (with lowest G-score value) was obtained from Glide and analysed.



General structure of docked ligand (1A to 15 A)

Table 1: Ligand structure details

LIGAND	R^1	R^2	IUPAC NAME
1	Cl	Cl	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-dichloro-1*H*-benzimidazol-2-yl) prop-2-en-1-one
2	F	F	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-difluoro-1*H*-benzimidazol-2-yl) prop-2-en-1-one
3	Br	H	(2*E*)-1-(5-bromo-1*H*-benzimidazol-2-yl)-4-(2- chloroquinolin-3-yl) but-2-en-1-one
4	O-CH_3	H	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-methoxy-1*H*-benzimidazol-2-yl) prop-2-en-1-one
5	F	H	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-fluoro-1*H*-benzimidazol-2-yl) prop-2-en-1-one
6	Cl	H	(2*E*)-1-(5-chloro-1*H*-benzimidazol-2-yl)-3-(2- chloroquinolin-3-yl) prop-2-en-1-one
7	Br	Br	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-dibromo-1*H*-benzimidazol-2-yl) prop-2-en-1-one
8	$COCH_3$	H	(2*E*)-1-(5-acetyl-1*H*-1,3-benzimidazol-2-yl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one
9	O-CH_3	O-CH_3	(2*E*)-3-(2-chloroquinolin-3-yl)-1-[5-methoxy-6- (methoxymethyl)-1*H*-benzimidazol-2-yl]prop-2-en-1-one
10	OH	OH	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-hydroxy-1*H*-1,3-benzimidazol-2-yl) prop-2-en-1-one
11	NO_2	H	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-nitro-1*H*-benzimidazol-2-yl) prop-2-en-1-one
12	CH_3	H	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-methyl-1*H*-benzimidazol-2-yl) prop-2-en-1-one
13	OH	H	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(4-hydroxy-1*H*-1,3-benzimidazol-2-yl) prop-2-en-1-one
14	NO_2	NO_2	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-dinitro-1*H*-benzimidazol-2-yl) prop-2-en-1-one
15	NH_2	NH_2	(2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-diamino-1*H*-1,3-benzimidazol-2-yl) prop-2-en-1-one

Table 2: Docking score against aromatase and EGFR

LIGAND	3S7S	3POZ
1A	-8.516	-8.112
2A	-8.286	-7.163
3A	-8.527	-7.122
4A	-7.945	-6.835
5A	-8.855	-6.799
6A	-8.055	-6.742
7A	-8.61	-6.474
8A	-8.339	-6.176
9A	-8.156	-5.703
10A	-9.878	-6.135
11A	-6.782	-5.48
12A	-8.314	-4.914
13A	-9.094	-6.646
14A	-8.316	-5.573
15A	-9.255	-4.706
Exemestane	−8.354	−8.066

3. RESULT AND DISCUSSION

The docking score of the proposed compounds against 3S7S and 3POZ is given in table 2.The docking score of reference exemestane was found to be -8.354 against 3S7S and -8.066 against 3POZ.The predicted binding pockets of aromatase comprise of the amino acids LEU 858, GLY 857, PHE 856, ASP 855, THR 854, CYS 775, LEU 775, LEU 788, LYS 745, THR 790, ALA743, LEU 792, MET 793, THR 790.The binding pockets of EGFR with PDB ID 3POZ comprise of the following aminoacids ARG 115, CYS 437, ALA 438, GLY 439, MET 303, GLU 302, ILE 133, ILE 132, LEU 152,VAL 370,LEU 372.

3.1 Docking against aromatase

As depicted in the table 2 dock score of the selected ligands are comparatively much higher than the exemestane. The values range from -9.878 to -6.782. The ligand 10A which is a dihydroxy substituted chalcone showed highest dock score of -9.878 with lower energy confirmation. The figure 1 shows the binding interaction of 10A with aromatase. The 2 hydroxy groups bind with the PHE 856 through hydrogen bonding interaction and the chlorine of the quinoline moiety interact with LYS 745.Ligand 15A showed dock score of -9.255 due to strong hydrogen bond interaction of both amino group with PHE 856 and keto group with LYS 745.Fig 2 shows the interaction of 15 A with aromatase. Ligand 13A with a hydroxy substituent gave a dock score of -9.094. Fig 3 depicts the binding of 13A in which the hydroxy group interact with PHE 856. Due the presence of hydroxy and amino groups it showed stronger polar interactions with the binding site.

3.2 Docking against EGFR

The dock score of ligands against 3POZ ranges from -8.112 to -4.706. Ligand 1A shows a stronger binding interaction with 3POZ with a dock score of -8.112. Fig 5 depict the interaction of ligand 1A with EGFR binding site. The keto group of the ligand bind with ALA 43 and a π-π interaction is seen between benzene and PHE 134.Similar interaction is seen in ligand 2A which is shown in fig 6. The dock score of 2A with difluoro substituent is -7.163. In ligand 5A interaction can be seen between the nitrogen of benzimidazole ring with ALA 438 and keto group of the chalcone with ARG 115 which is shown in fig no 4.

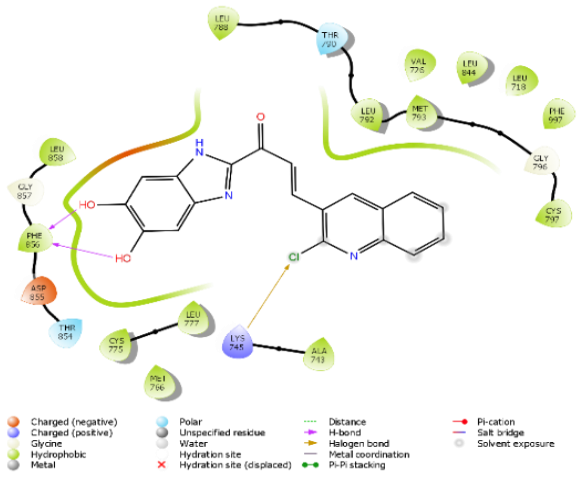


Figure 1: Interaction of 10A with aromatase

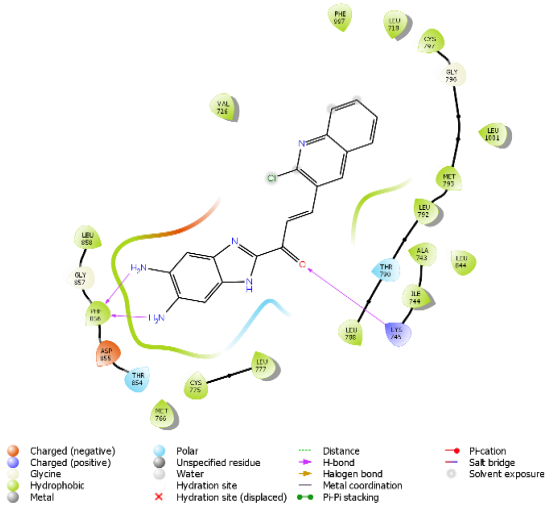


Figure 2: Interaction of 15A with aromatase

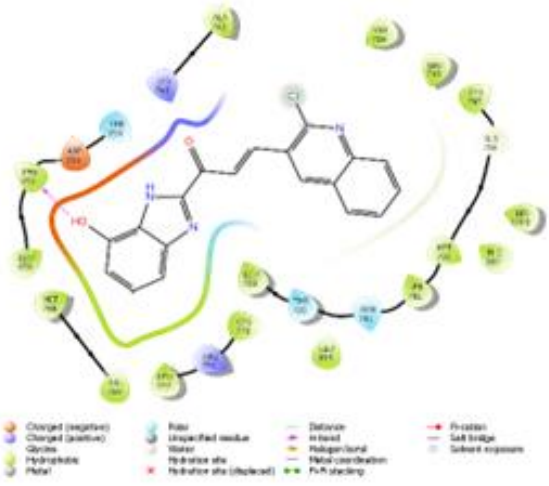


Figure 3: Interaction of 13A with aromatase

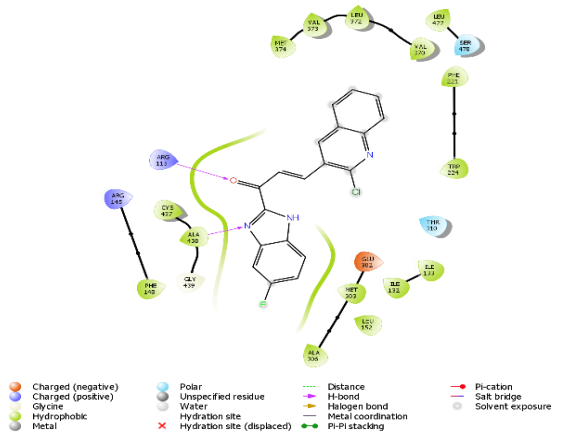


Figure 4: Interaction of 5A with EGFR

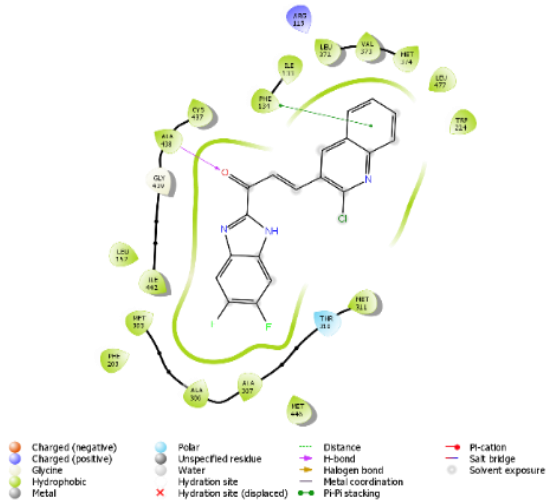


Figure 5: Interaction of 1A with EGFR

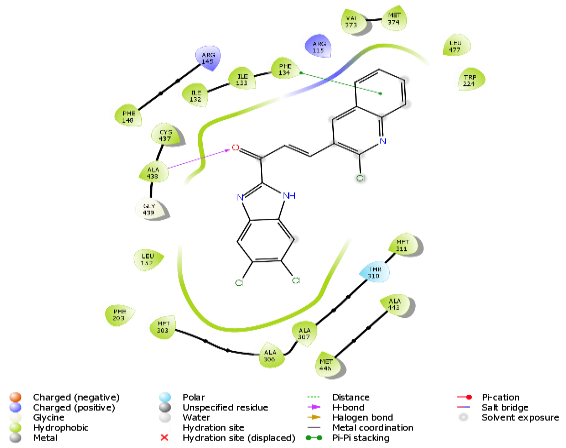


Figure 6: Interaction of 2A with EGFR

4. DISCUSSION AND CONCLUSION

The docking studies of novel chalcones comprising of quinoline and benzimidazole were carried out against aromatase (PDB ID 3S7S) and EGFR (PDB ID 3POZ). From the docking result it can be analysed that fewer derivatives have shown better docking score than exemestane. Majority of the compounds binds with aromatase with greater affinity than the standard inhibitor. The result shows a greater inhibitory activity for compound 10A and 15A against aromatase enzyme. The hydrogen bonding and electron release via resonance of these moiety play an important role in its receptor binding with 3S7S binding site.

The dock score of the ligands against 3POZ shows that they bind with the EGFR receptor site with lower interaction as compared to aromatase enzyme. The electron withdrawing nature of the dichloro derivative showed a better binding with 3POZ binding site. Greater electron withdrawing substituent like difluoro and electron donating substituents showed lower interaction with the target 3POZ.The overall energy and binding interactions shows that these novel moieties could be a better target for breast cancer therapy due to its inhibitory activity against both aromatase and EGFR which are the main enzyme targets involved in breast cancer development.

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