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IN SILICO ANALYSIS OF THE INHIBITORY ACTIVITIES OF NOVEL AZO DERIVATIVES OF BENZIMIDAZOLE ON EGFR (HER-2) KINASE DOMAIN

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ABSTRACT

Background: Epidermal growth factor receptors (EGFR) in human were involved in various types of cancers represented by abnormal signal transduction. This class consists of EGFR (ErbB1), HER2 (ErbB2, HER2), HER3 (ErbB3), and HER4 (ErbB4). Among them, EGFR and HER2 are related to breast cancer and are appropriate targets in dealing with various breast cancer cases. Disturbance of EGFR signalling, both by blocking EGFR binding sites or suppressing intracellular tyrosine kinase activity, can inhibit the rise of EGFR expressing tumours and recover the patient's condition. In this interest, a set of some new azo benzimidazole derivatives were prepared by coupling the diazonium derivative of benzimidazole with different suitable aromatic compounds and are used as ligands to dock against human HER2 kinase domain receptor. **Materials and Methods**: For this purpose, the crystal structure of the EGFR Kinase domain associated with TAK 285 (PDB ID: 3POZ, 1.5 Å X-ray resolution) was retrieved from the RCSB Protein Database (PDB) and used as the target. **Results**: All the compounds firmly inhibited by completely filling the active sites in the model with low energy values. **Conclusion**: Present study backed the in vitro activity of compounds designed by Mohanty et al., (4) and proved that the compounds of the current study inhibit the EGFR Kinase domain. So, these designed compounds can be used in drug development against some diseases that may somehow be related to the protein EGFR Kinase domain.

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INTRODUCTION

Breast cancer is the highest recurring cancer observed in women, affecting 2.0 million women every year, and accounts for the maximum number of cancer associated deaths in women. In the year 2019, it was calculated that around 688,562 women deceased because of breast cancer which is nearly 15% of all deaths related to cancer in women (1). Breast cancer rates are greater amid women in the developed countries; cases are growing in nearly every region universally. According to American cancer society, in 2021, it is estimated that 281,550 new cases of migrating breast cancer will be detected in women (2) and nearly 2,650 cases are estimated to be diagnosed in men (3).

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Benzimidazole is a fused heterocyclic organic compound structurally similar to nucleotides of human body which makes it an important molecule in medicinal chemistry. The benzimidazole nucleus is gathering importance in various research in medicinal chemistry, and many marketed drugs containing benzimidazole nucleus, produces efficient biological activities such as antitubercular, anticancer and antiviral (4). Present study is aimed to determine the inhibitory activities of some experimentally designed compounds on human Epidermal growth factor receptor (EGFR). EGFR is a kinase protein which gets activated upon binding of epidermal growth factor and Transforming growth factor (TGF)-α and both the endogenous ligands promotes growth of cells. Studies have revealed that EGFR is expressed & involved in prognosis of breast cancer(5). Current study is designed to check various interactions of prepared ligands and their effectiveness on the receptor.

In the present study we have considered human EGFR kinase domain 3D structure as target against a set of azo benzimidazoles, which were prepared by coupling diazonium derivative of benzimidazole with suitable aromatic compounds(6). The exact configuration of ligands with receptor binding site were analysed by careful docking of each ligand to the respective receptor (7).

MATERIALS AND METHODS

Ligand Dataset: Mohanty *et al.*, reported a set of novel azo benzimidazoles, which were prepared by coupling diazonium benzimidazoles with various suitable aromatic compounds, these compounds were used in docking studies against the receptor. Fig. 1 represents structures and IUPAC names of this series and named as compounds 6a (1–5) & 6b (1–5).

6a1(E)-5-(3-(2-(1H-benzo(d)imidazol-2-yl)-4-iodophenyl)triaz-1-en-1-yl)-2-hydroxybenzaldehye;
6a2 (E)-4-(3-(2-(1H-benzo(d)imidazol-2-yl)-4-iodopheny)triaz-1-en-1-yl)-2,6-dinitrophenol;
6a3 (E)-4-(3-(2-(1H-benzo(d)imidazol-2-yl)-4-iodophenyl) triaz-1-en-1-yl)-2,6-dichlorophenol;
6a4 (E)-8-(3-(2-(1H-benzo(d)imidazol-2-yl)-4-iodophenyl) triaz-1-en-1-yl)-5-hydroxy-2H-chromen-2-one;
6a5 (E)-4-(3-(2-(1H-benzo(d)imidazol-2-yl)-4-iodophenyl) triaz-1-en-1-yl)-2-hydroxybenzoic acid;
6b1 (E)-5-(3-(3-(1H-benzo(d)imidazol-2-yl)-5-bromophenyl) triaz-1-en-1-yl)-2-hydroxybenzaldehyde;
6b2 (E)-4-(3-(3-(1H-benzo(d)imidazol-2-yl)-5-bromophenyl) triaz-1-en-1-yl)-2,6-dinitrophenol;
6b3 (E)-4-(3-(3-(1H-benzo(d)imidazol-2-yl)-5-bromophenyl) triaz-1-en-1-yl)-2,6-dichlorophenol;
6b4 (Z)-(3-(3-(1H-benzo(d)imidazol-2-yl)-5-bromophenyl) triaz-1-en-1-yl)-5-hydroxy-2H-chromen-2-one;
6b5 (Z)-5-(3-(3-(1H-benzo(d)imidazol-2-yl)-5-bromophenyl)t riaz-1-en-1-yl)-2-hydroxybenzoic acid. Structure Prediction:

The crystal structure of EGFR Kinase domain complexed with TAK 285 (PDB ID: 3POZ, 1.5 Å X-ray resolution) was obtained from the RCSB Protein Database (PDB). Three-dimensional structure of human EGFR kinase domain (PDB ID: 3POZ) is known experimentally(8) and is shown in Fig. 2. The structural analysis of the protein was performed by using MGL tools(9), model optimization and validation was done by using Ramachandran plot(10),Procheck(11), and Errat(12)tools. Present protein with single chain A and sequence length of 327 was selected(13), which is presented in Fig. 3.

Molecular Docking: Automated dockings were performed by using various tools of Auto Dock 4.2,for determining the conformations of designed ligands (azo benzimidazoles) binding to the selected site of 3POZ(14)(15). Polar hydrogen atoms along with Kollman charges were added to the receptor proteins. All the non-polar hydrogen atoms present in the ligands, were fused followed by allotment of Gasteiger partial charges. Rotations were enabled for the bonds present in all the ligands, in this procedure, random orientations and torsions were also used. Grid maps were optimized by using Auto grid carefully. Docked conformation having lowest binding energy was identified for every selected ligand.

Active binding residues: With the help of PDB (ID: 3POZ), all the active site residues present in the receptor was identified and docking studies were carried out.

Residues extracted: Val 726, Lys 745, Leu 788, Thr 790, Arg 841, Asn 842, Leu 844, Thr 854.

RESULTS AND DISCUSSION

Structure validation: Crystal structure of 3POZ (PDB ID: 3POZ; resolution 1.5 A˚) was used. Ramachandran plot represented 99.6% residues in the favourable regions and none in the unfavourable portion. In addition to this the other important parameters such as peptide bond planarity, tetrahedral distortion, non-bonded interactions, main chain H bond energy and total G factor for the selected structure are present inside the allowed range. The homology models were confirmed by the help of Errat tool. The total quality factor for non-bonded atomic interactions was well high above the allowed range i.e., 98.8604 which reflected the quality of the selected model. Figure 3 represents the structure verification by Ramachandran plot, Errat programs and Pro check. All the above data verified that the selected model is of high quality and can be used for performing further studies.

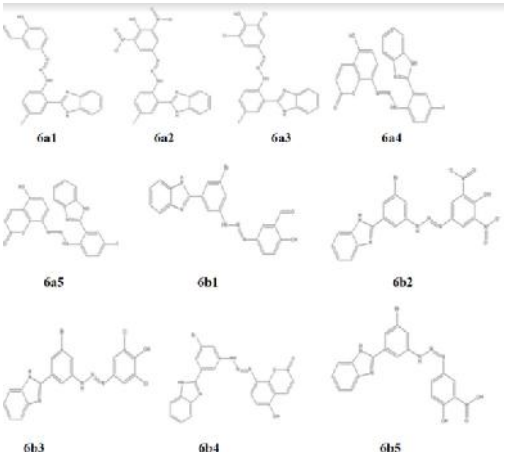

Figure 1. 2D Structure of azo derivatives of benzimidazole and their IUPAC names (6a1-5 and 6b1-5)

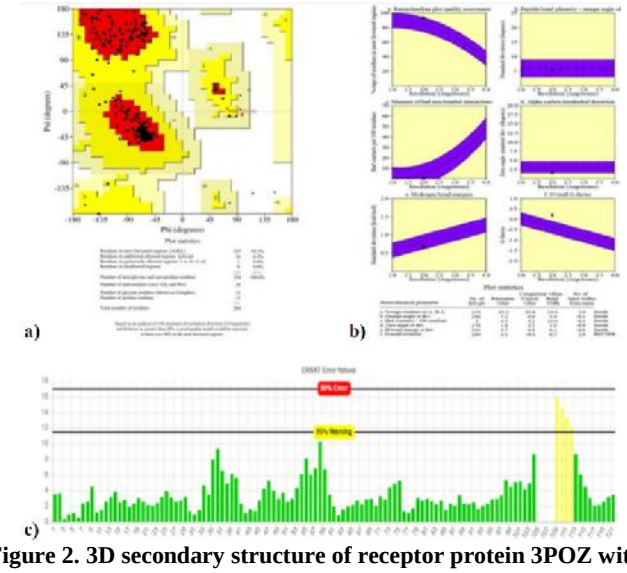

Figure 2. 3D secondary structure of receptor protein 3POZ with resolution of 1.5 A˚

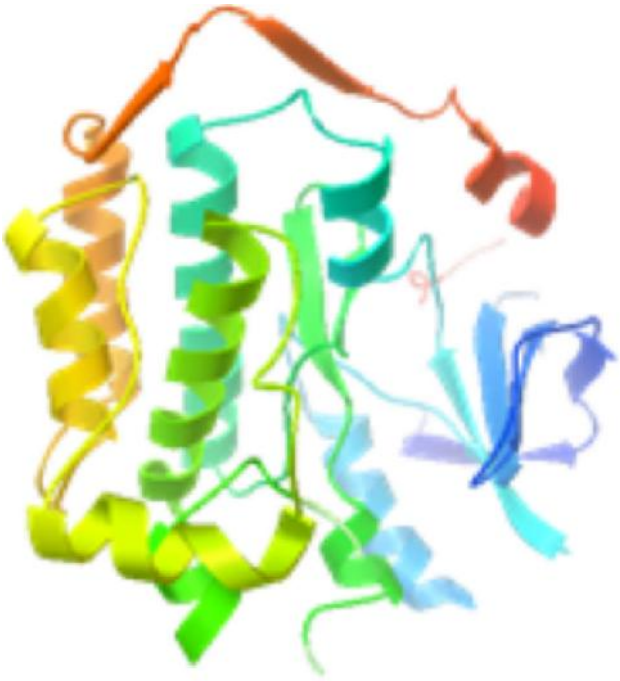

Figure 3. Structural validation of receptor protein, 3POZ. (a) Ramachandran plot representing 99.6% residues in the favourable region; (b) Procheck validation plot for active chain parameters; (c) Errat quality check graph with total quality factor of 98.8604

Molecular dockings: Docking studies were performed with the receptor protein. All the selected ligands produced low binding energy values as represented in Table-1. The potency of the ligands to inhibit the target, importantly the compounds 6a2, 6a3, 6b3, 6b4 and 6b5 were proved by their energy values. The binding energy values were found to be in the range between -9.0 to -11.5 kcal / mol. The H bond energy values were found to be in the range between -0.903 to -3.844 kcal/mol. Moreover, the intermolecular energy values, Hydrogen bond and electrostatic energy values were also found to be in acceptable limit for the selected protein represented in Table-1 and Fig. 4.

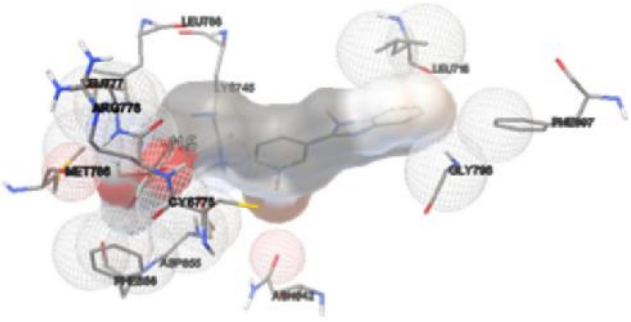

Figure 4. Binding of all ligands in receptor protein, 3POZ

Binding Mode of Azo Derivatives of Benzimidazole: All azo derivatives of benzimidazoles bound proficiently with active binding sites of receptor protein, 3POZ. Fig 4represents the binding pose of the compounds with 3POZ protein. It is clearly understood that all compounds occupied the same binding site in the protein. Fig. 5 further represents the residues that are involved in binding with the receptors. Fig 5 shows that Lys 745, Leu 788, Asn 842 residues of protein is involved in interaction. Receptor protein, 3POZ interaction with the compounds 6a1-6a5 and 6b1-6b5 was studied carefully in detail. Table 2 shows the binding residues reports for ligand 6a1-6a5 and 6b1-6b5. It was analysed that all active site residues for target protein, 3POZ were engaged in docking with all the ligands. Table 3 and Fig 6 shows the hydrogen bonds and bond distance of the compounds and target protein residues. Compounds 6a4 and 6b2 each make two hydrogen bonds with two residues. Asn 842, Lys 745 and Thr 854 of 3POZ protein were observed in forming hydrogen bonds with these ligands, but Asn 842 was the most repeatedly visible residue that made bonding with hydrogen. Table 3 represents hydrogen bonds atoms involved and bond length. Fig. 6 represents the reacting residues engaged in docking of ligands at the active site of 3POZ protein. Compounds 6a1, 6a5, 6b3, and 6b4 form one hydrogen bonds with Asn 842, Arg 841, Lys 745 and Thr 790 as shown in the Table 3. Compounds 6a2, 6a3, 6b1 and 6b5 do not form any hydrogen bond with any of the residues of the receptor protein, 3POZ.

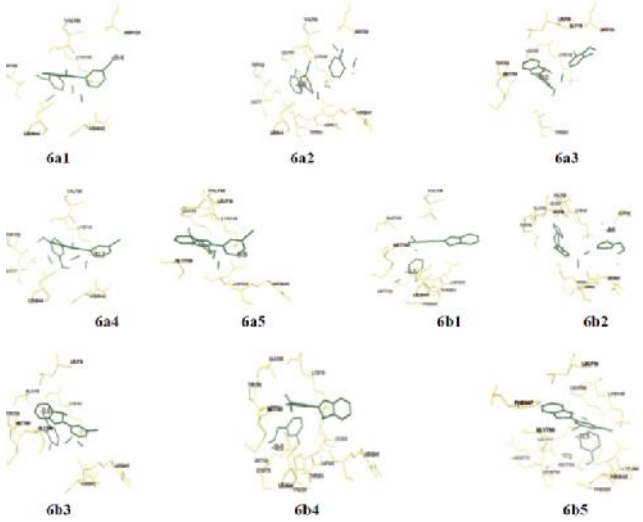

Figure 5. Various residues engaged in binding with target protein. All the compounds are represented in sticks encircled by the mesh surface. Target residues are represented in sticks

Compound 6a1 forms one hydrogen bond with Asn 842 (2.144 A˚), while it undergoes hydrophobic interaction with Val 726, Lys 745, Thr 790 and Leu 844 as shown in the Fig 6a. Compound 6a2 forms no hydrogen bonds and only hydrophobic interaction with Val 726, Lys 745, Leu 788, Thr 790, Arg 841, Leu 844 and Thr 854 (Fig. 6b). Compound 6a3 also forms no hydrogen bonds and interact hydrophobically with Lys 745, Leu 788, Thr 790 and Thr 854 (Fig 6c). Compound 6a4 forms two hydrogen bonds with Asn 842 (2.22 A˚) and Lys 745 (2.111 A˚) and interact hydrophobically with Val 726, Thr 790 and Leu 844 (Fig 6d). Compound 6a5 forms one hydrogen bond with Arg 841 (2.037 A˚) and undergoes hydrophobic interaction with Val 726, Lys 745 and Leu 788 (Fig 6e). Compound 6b1 forms no hydrogen bonds and undergoes hydrophobic interaction with Val 726, Leu 844 and Thr 854 (Fig 6f). Compound 6b2 forms two hydrogen bonds with Asn 842 (1.925 A˚) and Thr 854 (2.158 A˚) and interact hydrophobically with Val 726, Lys 745, Leu 788, Thr 790 and Arg 841 (Fig 6g). Compound 6b3 forms one hydrogen bond with Lys 745 (2.813 A˚), while it interacts hydrophobically with Thr 790, Arg 841 and Asn 842 (Fig 6h). Compound 6b4 forms one hydrogen bond with Thr 790 (1.919 A˚), while it interacts hydrophobically with Lys 745, Arg 841, Leu 844 and Thr 854 (Fig 6i). Compound 6b5 forms no hydrogen bonds and interact hydrophobically with Lys 745, Leu 788 and Asn 842 (Fig 6j).

Table 1. Docked energy values of all ligands with target protein, 3POZ

S.No	Compds.	Binding Energy (kcal/mol)	Hydrogen bond energy (kcal/mol)		Hydrogen bond energy (kcal/mol)	Torsional free energy (kcal/mol)
			Ideal value	Std. deviation		
1	6a1	-9.4	-2.0	0.8	-1.85	1.00
2	6a2	-11.5	-2.0	0.8	-	1.00
3	6a3	-11.0	-2.0	0.8	-	1.00
4	6a4	-10.3	-2.0	0.8	-1.306	1.00
5	6a5	-9.5	-2.0	0.8	-2.501	1.00
6	6b1	-9.8	-2.0	0.8	-	1.00
7	6b2	-9.5	-2.0	0.8	-3.844	1.00
8	6b3	-11.6	-2.0	0.8	-0.903	1.00
9	6b4	-10.6	-2.0	0.8	-1.323	1.00
10	6b5	-10.5	-2.0	0.8	-	1.00

Table 2. Receptor target, 3POZ, active site residues engaged in docking interactions with the ligands

Compds.	Residues involved in binding							
	VAL 726	LYS 745	LEU 788	THR 790	ARG 841	ASN 842	LEU 844	THR 854
6a1	✔	✔		✔		✔	✔	
6a2	✔	✔	✔	✔	✔		✔	✔
6a3		✔	✔	✔				✔
6a4	✔	✔		✔		✔	✔	
6a5	✔	✔	✔		✔			
6b1	✔						✔	✔
6b2	✔	✔	✔	✔	✔	✔		✔
6b3		✔		✔	✔	✔		
6b4		✔		✔	✔		✔	✔
6b5		✔	✔			✔		

Table 3. Atoms of the ligands and receptor residues involved in forming hydrogen bonds and bond length

S.No	Compounds	No. of H bonds	Binding Residue	H bond	Distance (A°)
1	6a1	1	ASN 842	O-HN	2.144
2	6a2	0	-	-	-
3	6a3	0	-	-	-
4	6a4	2	ASN 842	O-HN	2.22
			LYS 745	H-N	2.111
5	6a5	1	ARG 841	O-HN	2.037
6	6b1	0	-	-	-
7	6b2	2	ASN 842	O-HN	1.925
			THR 854	O-HN	2.158
8	6b3	1	LYS 745	H-N	2.813
9	6b4	1	THR 790	H-O	1.919
10	6b5	0	-	-	-

Summary

Mohanty *et al.* narrated a set of new azo benzimidazoles, which was synthesized by coupling diazo benzimidazoles with various suitable aromatic compounds (5a1-5 and 6a1-5) and analysed their potencies by invitro activities. Present work deals in the *in-silico* analysis of these derivatives against human HER2 kinase domain receptor. For this purpose, the crystal structure of EGFR Kinase domain complexed with TAK 285 (PDB ID: 3POZ, 1.5 Å X-ray resolution) was retrieved from the RCSB Protein Database (PDB) and used as target. Ramachandran plot, Procheck, and Errat tools were used for protein structure prediction, model optimization and validation for meticulous docking studies. Most compounds strongly inhibited the target protein by totally binding with the active sites present in the proteinwith minimum energy values. The binding values reflected the potency of the ligands to inhibit the target mostly the compounds **6a2, 6a3, 6b3, 6b4** and **6b5**. The binding energy values range from -9.0 to -11.5 kcal/mol. The Hydrogen bond energy values were found to be in the range from -0.903 to -3.844 kcal/mol. Moreover, the intermolecular energy values + H bond + electrostatic energy values were also found in a favourable range for the selected receptor protein.

Conclusion

This present *insilico* analysis of the prepared ligands supports the in-vitro anticancer activity published by Mohanty *et al.* (2018) and thus provesblocking activity on EGFR Kinase domain that can be used in the treatment of breast cancers. Inhibitory binding potencies of the ligands in the active site residues with potent activity, strong binding, low energy values and inhibition values revealed that these compounds are active scaffolds and can be used in drug design against specific diseases that can somehow be linked to the protein EGFR Kinase domain.

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Conflict of interest: The authors of this paper have no conflicts of interest to declare.

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