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Molecular Modeling of Some Benzodiazole Derivatives with EGFR Protein 1M17

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Authors' contributions

This work was carried out in collaboration between both authors. Author ASA designed the study, performed the analysis, wrote the protocol and wrote the first draft of the manuscript. Author XS managed the analyses of the study managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the anticancer potential of benzodiazole derivatives using molecular modeling studies.
Study Design: Molecular Dynamics simulation study.
Place and Duration of Study: Sathyabama Institute of Science and Technology (SIST), Chennai, between June 2020 and August 2020.
Methodology: We studied the anticancer potential of benzodiazole derivatives using molecular modeling. Docking studies of the ligands with EGFR protein 1M17 was carried out using AutoDock.Molecular Dynamics simulation study was carried out using Playmolecule was used to verify the stability of the protein-ligand complex.
Results: Molecular docking studies showed a good binding affinity of the ligands with the protein 1m17. Benzodiazole derivative 4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole exhibited the lowest binding energy of (-6.42 kcal/mol) at the active site of EGFR (PDB code:1M17) consistent with its least inhibition coefficient (Ki =32.54 uM). Molecular dynamics simulation showed better stability of the ligand and protein complex.

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Conclusion: Molecular modeling study of selected benzodiazole derivatives showed a very good binding affinity to EGFR protein 1m17. MD simulation of the best-docked ligand showed that the complex was stable. Our study demonstrated that benzodiazole derivatives can be potential anticancer drug candidates

Keywords: Anticancer; heterocyclic; docking, molecular dynamics simulation; benzodiazole.

1. INTRODUCTION

Cancer is a global health problem, affecting the world population irrespective of socioeconomic status or regional differences. Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018 [1]. Lung, prostate, colorectal, stomach, and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical, and thyroid cancer are the most common among women (WHO). Cancer research has revealed around 1000 different potential agents to cause cancer [2]. Though cancer was prevalent on earth centuries before, the disease remained mysterious till the beginning of the twentieth century [3].

Among various types of cancer lung cancer is the commonest cancer among men. Epidermal growth rate factor (EGFR) is frequently expressed in non-small cell lung cancer [4]. EGFR-positive lung cancer refers to lung cancers that show evidence of an EGFR mutation. EGFR, or epidermal growth factor receptor, is a protein present on the surface of both healthy cells and cancer cells. Mutated EGFR doesn't perform the way it should. Instead, it causes rapid cell growth, helping cancer spread. A protein found on certain types of cells that binds to a substance called an epidermal growth factor. The EGFR protein is involved in cell signaling pathways that control cell division and survival. Sometimes, mutations in the EGFR gene cause EGFR proteins to be made in higher than normal amounts on some types of cancer cells. This causes cancer cells to divide more rapidly. Drugs that block EGFR proteins are being used in the treatment of some types of cancer. The epidermal growth factor receptor (EGFR) is commonly overexpressed in cancers such as non-small-cell lung cancer, metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, and breast cancer [5]. Aberrant expression of epidermal growth factor receptor can promote cell proliferation and tumorigenesis [6]. The present study explores molecular modeling and docking studies of benzodiazole analogs on EGFR proteins.

The discovery of the EGFR receptor tyrosine kinase inhibitors like gefitinib and erlotinib resulted in a large scale clinical trial of advanced-stage lung cancer patients [7]. Activation of the EGFR stimulates cellular growth, proliferation, invasion, and metastasis and inhibits apoptosis. Therefore, blockade of EGFR mediated effects should theoretically arrest the growth of nonsmall-cell lung carcinoma (NSCLC) driven by EGFR signaling.

2. MATERIALS AND METHODS

2.1 Softwares and Tools

Marwin Sketch, LigPlot, Protein Data Bank (PDB), PubChem and Playmolecule.

2.2 Ligand Preparation

The benzodiazole analogs for specific proteins were used as ligands for docking studies Table 1. The ligands were chosen based on their specific modes of action on cancer receptors. A molecular dynamics tool Playmolecule program was used for the molecular modeling using appropriate energy minimization steps and simulations previously described. The ligand molecules were drawn in either Marwin Sketch freeware saved as MDL molfile formats. The interaction of ligands used in the study are represented in Table 2.

2.3 Protein Preparation

The protein structures were obtained from the protein data bank (PDB). The proteins selected for the study were epidermal growth factor's receptor kinase domain (EGFR; PDB ID: 1m17). The files in PDB format for each receptor were converted to the respective PDBQT format using MGL tools. The polar hydrogen atoms were added to the receptor molecules before docking studies.

A series of 6 ligand molecules were taken from the literature [8]. While none of the compounds were novel, molecules with benzodiazole derivatives with substituents at the 1-, 2- and 5-positions.

Table 1. Details of the ligands

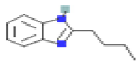
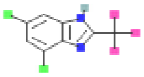
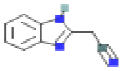
No.	LIGANDS	SMILES	MOLECULAR STRUCTURE
1	2-butyl-1H-1,3-benzodiazole	CCCCC1=NC2=CC=CC=C2N1	
2	1H-1,3-benzodiazol-4-amine	C1=CC(=C2C(=C1)NC=N2)N	
3	5,6-dimethyl-1H-1,3-benzodiazole	CC1=CC2=C(C=C1C)N=CN2	
4	4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole	C1=C(C=C(C2=C1NC(=N2)C(F)(F)F)Cl)Cl	
5	5,6-dimethyl-2-(trifluoromethyl)-1H-1,3-benzodiazole	CC1=CC2=C(C=C1C)N=C(N2)C(F)(F)F	
6	2-(1H-1,3-benzodiazol-2-yl)acetonitrile	C1=CC=C2C(=C1)NC(=N2)CC#N	

Table 2. Decomposed interaction energies of the ligand in kcal/mol

S.No.	Ligand	Est. Free energy of binding	Est. inhibi. constant,Ki(uM)	vdW+ Hbond+ desolv Energy	Electrostatic Energy	Total Intermolec Energy
1	5,6-dimethyl-1H-1,3-benzodiazole	-5.34	128.58	-5.31	-0.03	-5.34
2	5,6-dimethyl-2-(trifluoromethyl)-1H-1,3-benzodiazole	-5.46	99.94	-5.73	0.03	-5.76
3	4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole	-6.12	32.54	-6.40	-0.02	-6.42
4	1H-1,3-benzodiazol-4-amine	-4.19	851.06	-4.22	-0.27	-4.49
5	1H 2-(1H-1,3-benzodiazol-2-yl)acetonitrile	-4.93	242.7	-5.49	-0.04	-5.53
6	2-butyl-1H-1,3-benzodiazole	-5.10	182.17	-5.88	-0.06	-5.94

Table 3. Molecular interactions of ligands with amino acids of proteins

S.No.	Ligand	Hydrophilic interactions	Hydrophobic Interactions	No. of H-Bonds
1	5,6-dimethyl-1H-1,3-benzodiazole	GLU738,ASP831	VAL702,LEU764, ALA719,MET742	2
2	5,6-dimethyl-2-(trifluoromethyl)1H-1,3-benzodiazole	THR766	LEU820,ALA719, ASP831,THR830, GLU738	1
3	4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole	THR766	MET769,THR830, GLN767,GLU738 LEU820,ALA719	
4	1H-1,3-benzodiazol-4-amine	LYS721, ASP831,THR766	VAL702,MET742	2
5	1H 2-(1H-1,3-benzodiazol-2-yl)acetonitrile	MET742,GLU738 THR830	LEU764,VAL702 ALA719 LEU820,LEU768	2
6	1H 2-(1H-1,3-benzodiazol-2-yl)acetonitrile		LEU694,ALA719 CYS751	

3. RESULTS AND DISCUSSION

3.1 Protein - Ligand Interaction

LigPlot was used to study protein-ligand interactions for a given PDB file-encrypting the docking. The LigPlot program self-generated schematic 2D representations of the interfaces of protein-ligand complexes from standard PDB file input.

3.2 Molecular Docking

The molecules selected were drawn using the Marwin tool and uploaded to a web-based database of Docking server [9]. EGFR protein of Im17 was downloaded from the protein data bank (PDB) [10]. The crystal structure of protein 1m17 is given in Fig.1. Docking simulations were performed using Auto Dock [11]. The Lamarckian genetic algorithm (LGA). Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. The results of the docking studies are given in Figs. 2-7. The protein-ligand interaction study was performed using LigPlot. The interactions of the ligands with amino acid residues of the target proteins are shown. The H-bonds and hydrophobic contacts between the docked complexes are shown in Figs. 8-13.

The output was in the form of an informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic contacts, and atom accessibilities. Hydrogen bonds are represented by dashed lines whereas hydrophobic contacts are depicted schematically. The amino acid residues of the protein involved with the above contacts are shown by an arc with spokes emerging towards the ligand atoms in contact and vice versa. Table 2 shows the interaction of the ligands studied with EGFR protein 1m17. Figs. 8-13 shows the 2D interaction plots of the ligands with the protein.

Ligands formed hydrogen bonds (H bonds) with the residues Asp 831, GLU738 in Fig 8. Thr766 in Fig. 9 and Thr766, Asp831 in Fig. 11. In addition to the hydrogen bonding interactions, the hydrophobic interactions with VAL702, LEU764, ALA719, AND MET742 were present in Fig. 8. Halogen bonding with ASP831, THR830, GLU738 was prominent in Fig 9. Fig 3 shows prominent halogen bonds with residues MET769, THR830, GLN767, GLU738. Figs. 3-5 shows a mixture of halogen, polar and hydrophobic bonds whereas Fig. 6. shows predominant hydrophobic bonds. The decomposition of binding energies of molecules studies is given in Table 2. ki - inhibition constant is correlated to the half of maximal inhibitory constant at which 50% of protein is inhibited. This indicates the amount of drug or substance needs to inhibit the biological process. Studied molecules showed the best binding energies against EGFR protein receptors. Derivatives exhibited the lowest binding energy of (-6.29 kcal/mol) at the active site of EGFR (PDB code:1M17)consistent with its least inhibition coefficient (Ki =32.54 uM). Our results demonstrated that the benzodiazole

derivatives studied have anticancer drug potential.

The best docking poses were selected considering the interaction energy between protein and ligand can be related to binding affinities.

3.2 Molecular Dynamics Simulation

Play Molecule [12] consists of a set of applications that can "talk to each other" this is, the output of one application can be used as input for another application. For instance, the System Builder application requires a prepared protein structure that can be obtained through the application Protein Prepare. Therefore, to build and run an MD simulation, we must use many applications in succession. The outline of the pipeline used is given in Fig. 14.

Protein Prepare was used to protonate and optimize a protein structure (PDB;1m17). The expected output is a protein structure with the most likely protonation state (at pH 7.4) and with an optimized hydrogen bond network. System Builder is an application that was used to run Molecular Dynamics (MD) simulations. This program generates systems for Amber Force Fields [13]. System Builder helps the length of the simulation and leave the rest of the options by default. In SimpleRun one can visualize the simulation and generate plots regarding the fluctuation of the ligand.

MD simulations of the ligand (4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole) exhibit best binding affinity (-6.42 kcal/mol) and least inhibition constant (32.54 uM) in the docking study. MD simulations were performed and the results were shown in the Fig. 15-16. From the root mean square deviation (RMSD) plot there is not much difference in the RMSD of the protein-ligand complex during the simulation. The complex formed is stable. The root mean square fluctuation (RMSF) trajectories of the complex showed lesser fluctuation most of the time. Therefore it can be assumed that the complex is more stable. This may be due to the higher number of hydrophobic bonds formed between the ligand and the protein [14].

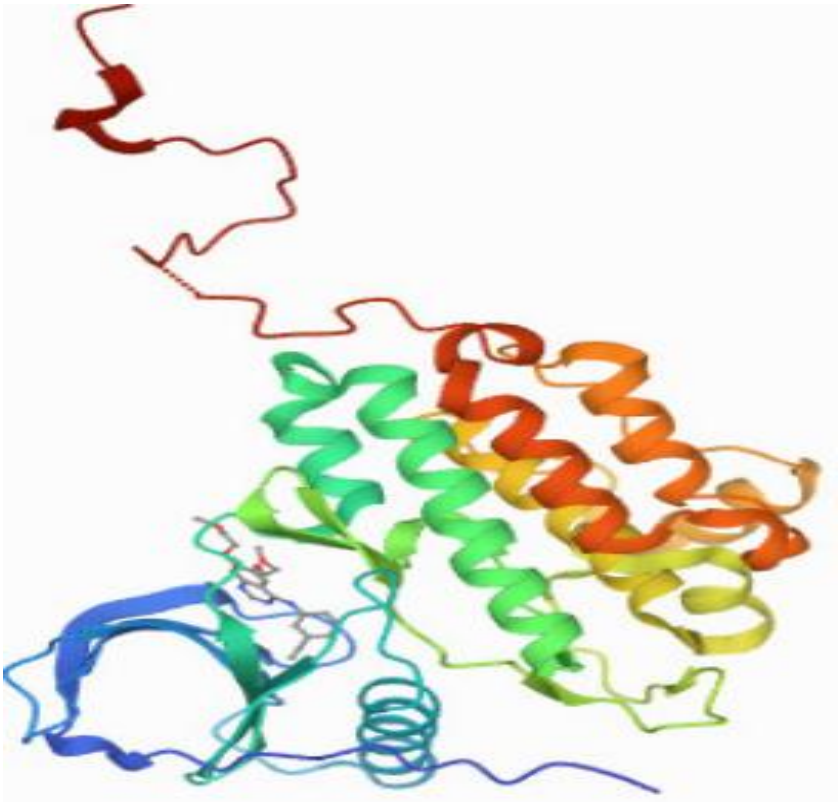


Fig. 1. Structure of EGFR protein 1M17

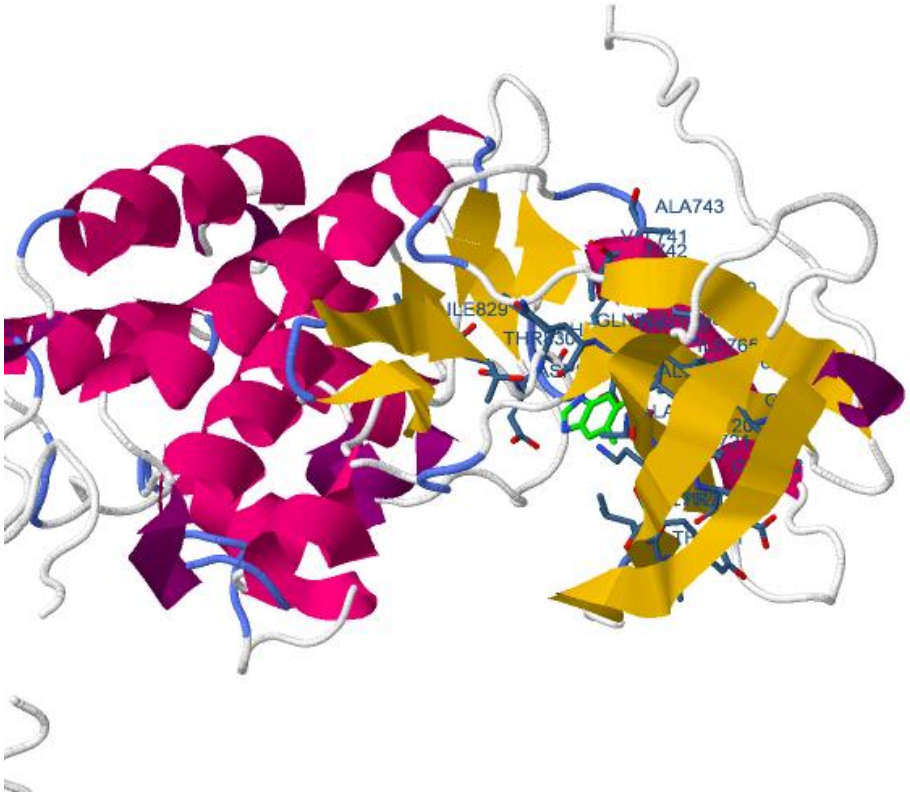


Fig. 2. (Docking of 5,6-dimethyl-1H-1,3-benzodiazole)

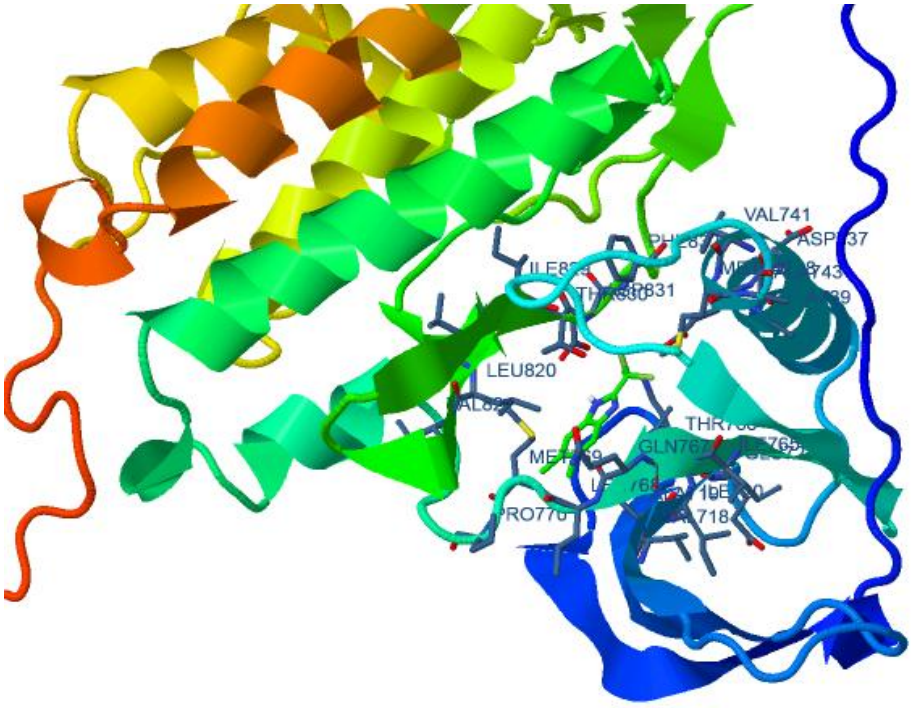


Fig. 3. (Docking of 4,6-dichloro-2-(trifluoromethyl)-1H-1,3-benzodiazole)

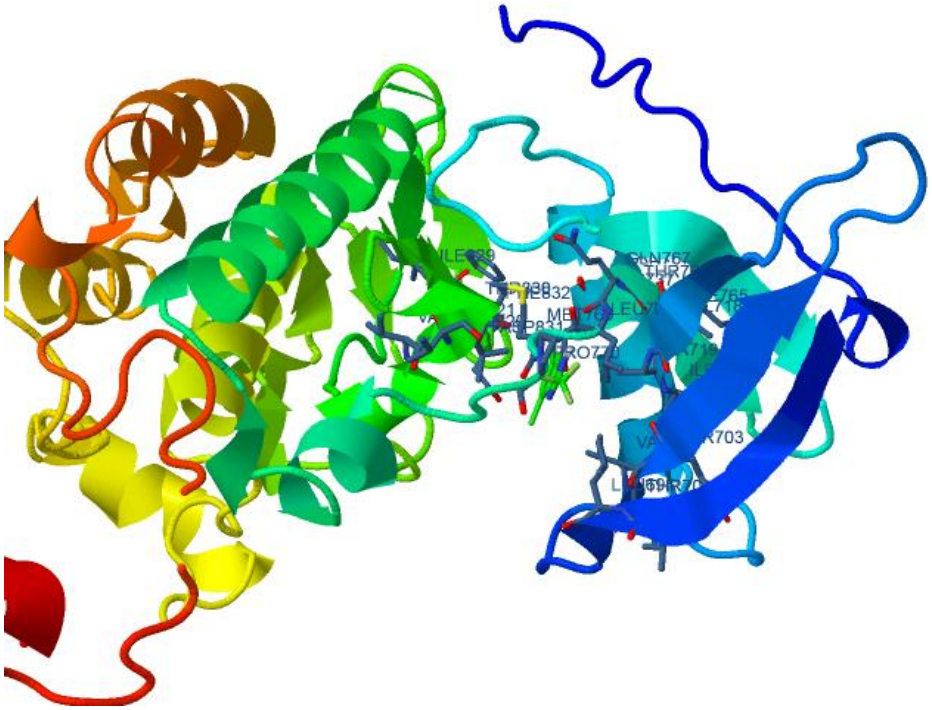


Fig. 4. (Docking of 1H-1,3-benzodiazol-4-amine)

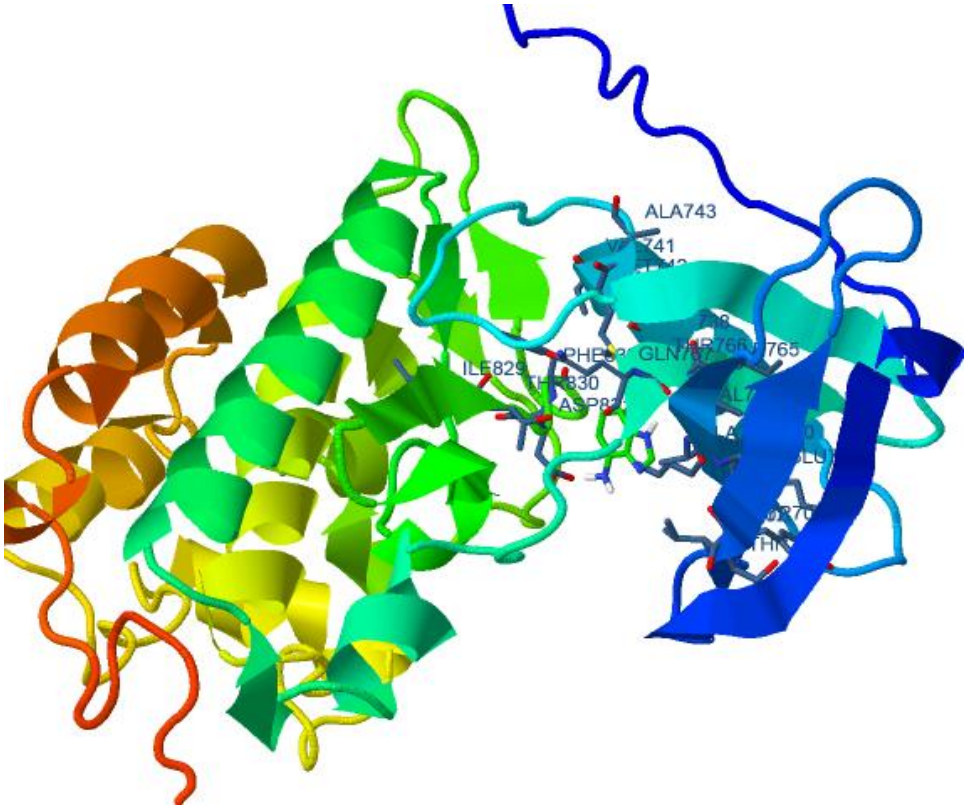


Fig. 5. (Docking of 2-(1H-1,3-benzodiazol-2-yl)acetonitrile)

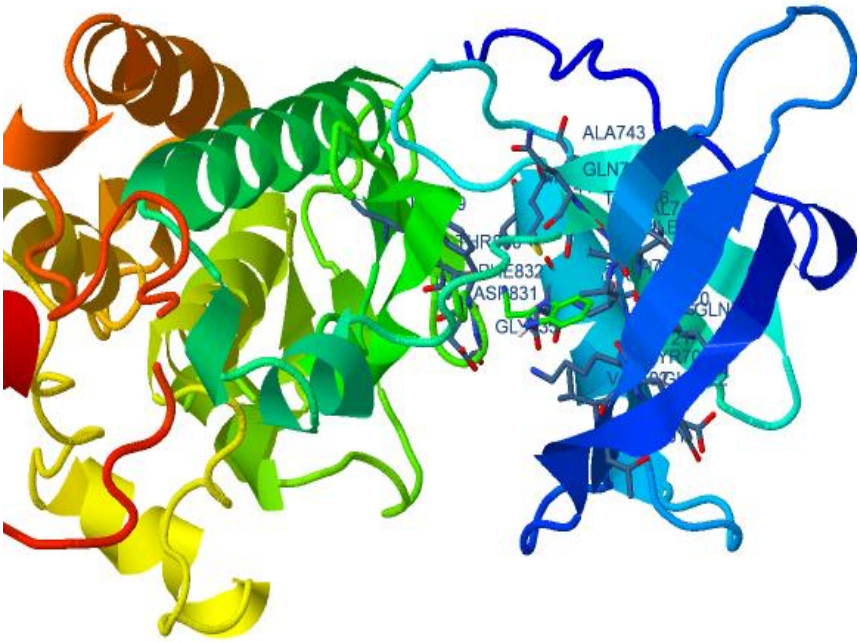


Fig. 6. (Docking of 2-butyl-1H-1,3-benzodiazole)

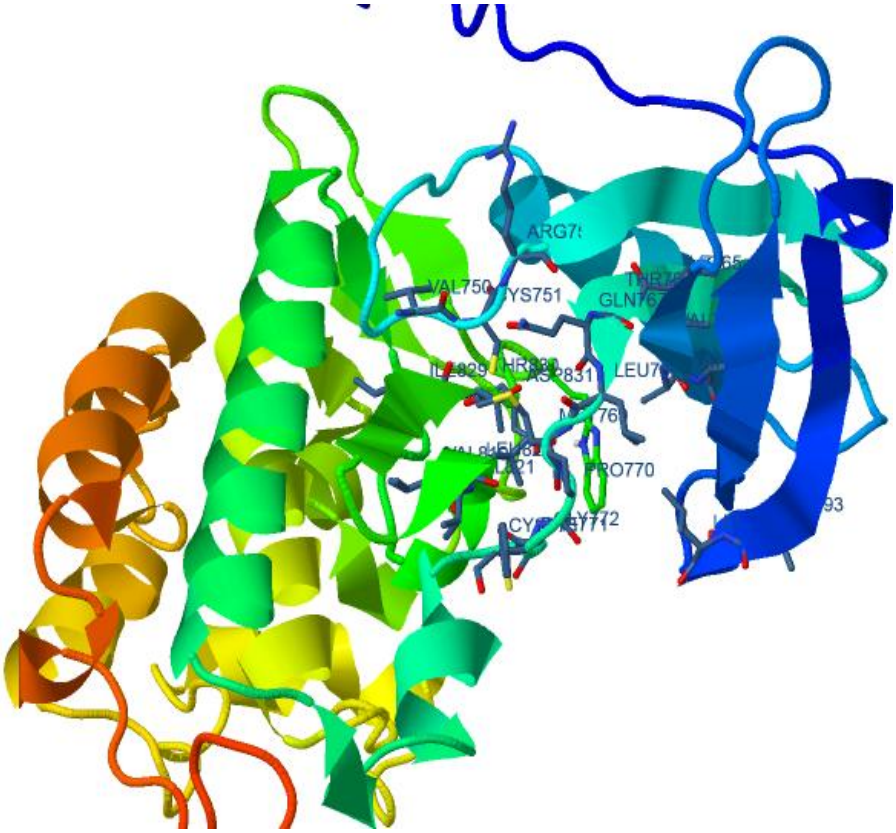


Fig. 7. (Docking of 4,6-dibromo-2-(trifluoromethyl)-1H-1,3-benzodiazole)

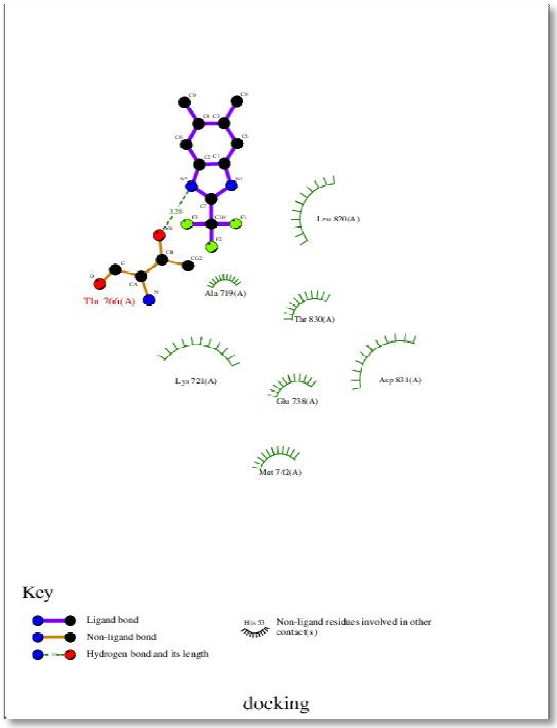


Fig. 8. 2D interaction Plot of Ligand 1

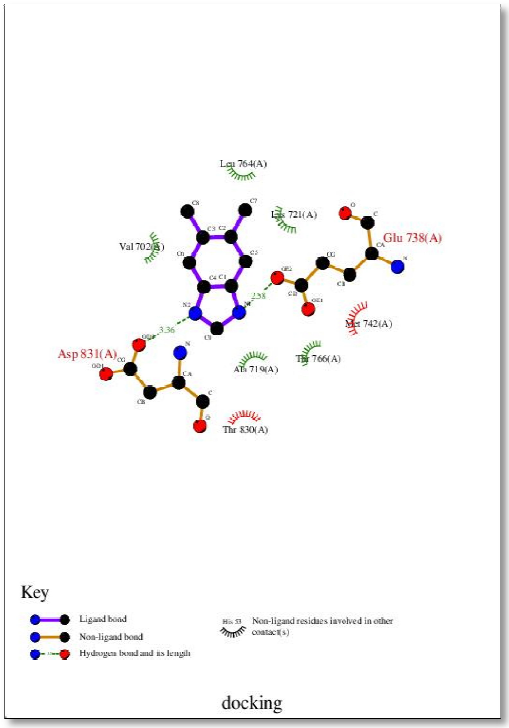


Fig. 9. 2D interaction Plot of Ligand 2

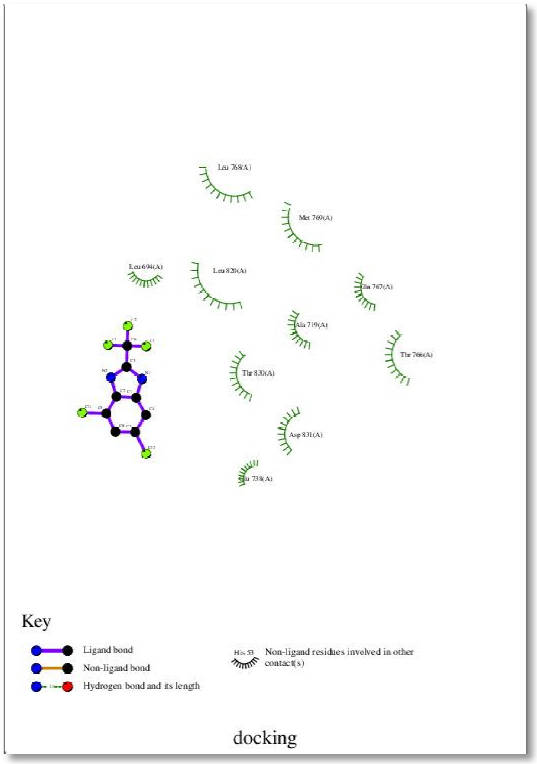


Fig. 10. 2D interaction Plot of Ligand 3

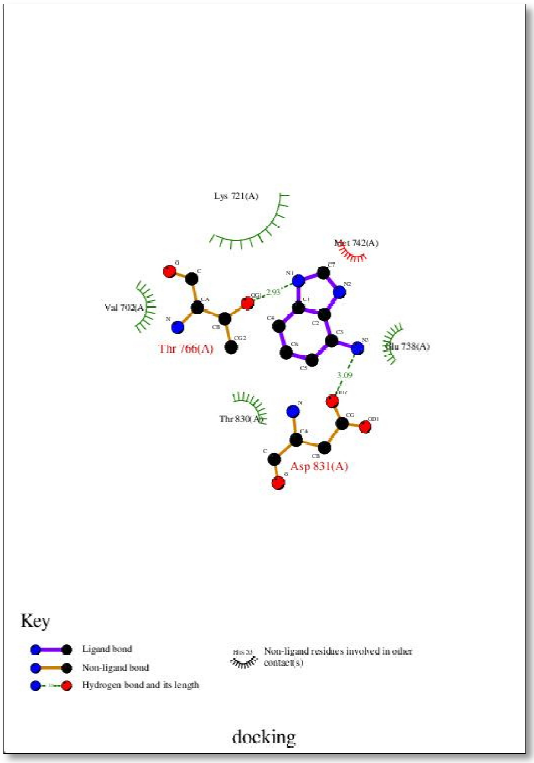


Fig. 11. 2D interaction Plot of Ligand 4

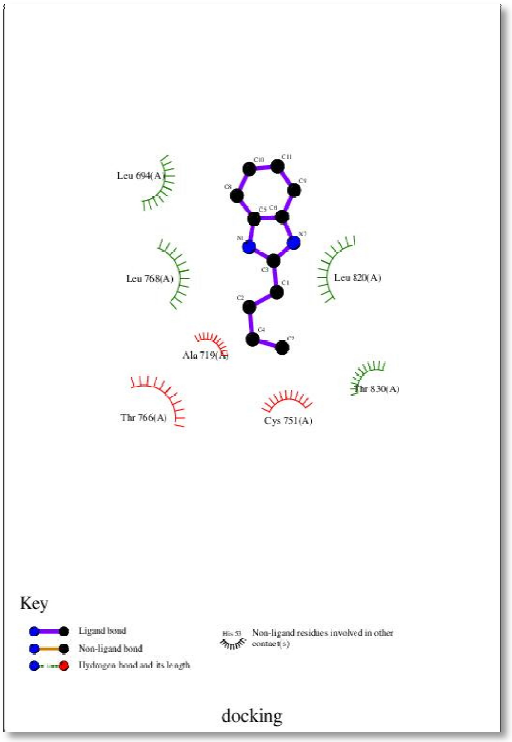


Fig. 12. 2D interaction Plot of Ligand 5

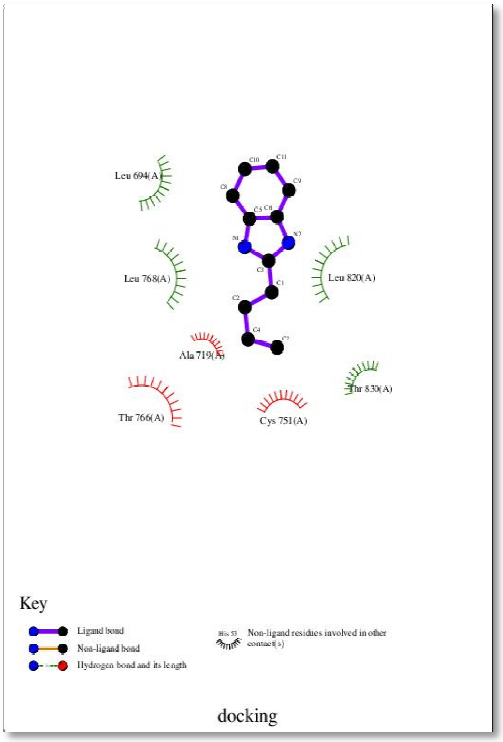


Fig. 13. 2D interaction Plot of Ligand 6

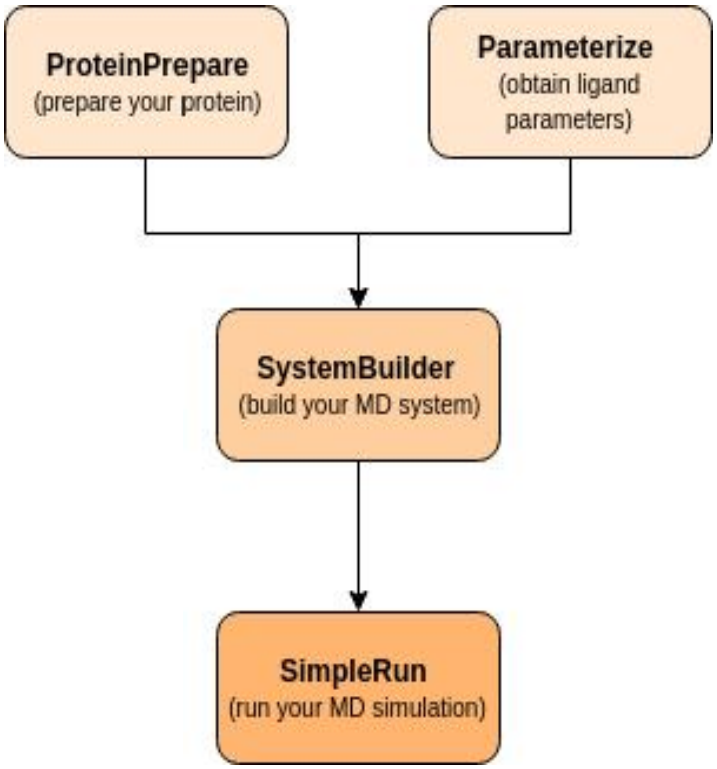


Fig. 14. Flowchart showing the pipeline of molecular dynamics simulation

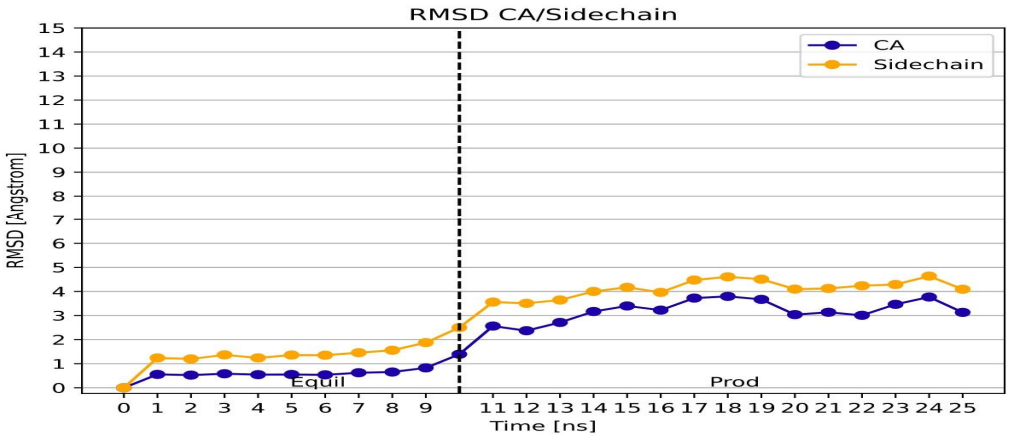


Fig. 15. RMSD of the protein-ligand simulation

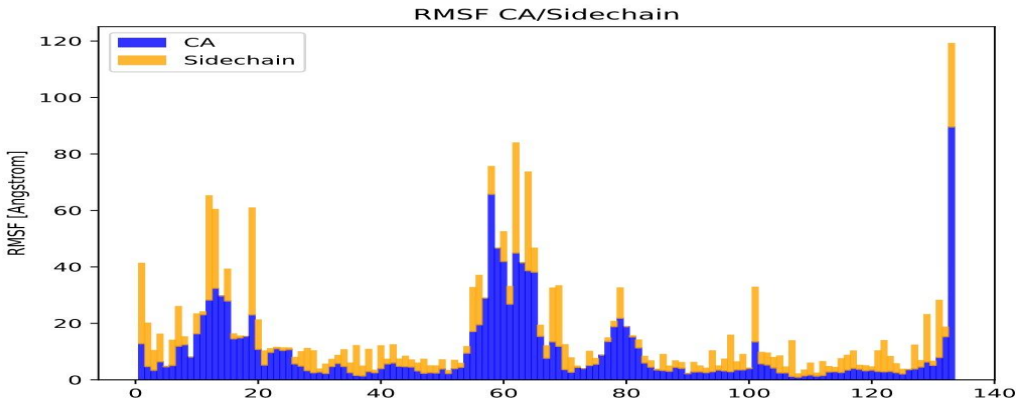


Fig. 16. RMSF of the protein-ligand simulation

4. CONCLUSION

Molecular docking studies of molecules showed the best binding energies against EGFR protein 1M17. Ligand which the lowest binding energy of -6.29 kcal/mol at the active site of EGFR (PDB code:1M17) consistent with its least inhibition coefficient (Ki =32.54 uM). The low inhibition coefficient indicates that the molecule possesses a high potential to be potential FGFR inhibitors. The best complex model of protein-ligand pair was used for molecular dynamics simulation. Molecular dynamics simulation data processed by calculating ligand RMSD to evaluate binding stability. MD simulation showed that the complex was stable during the simulation. Our results demonstrated that proposed benzodiazole derivatives showed significant anticancer drug characteristics and may be potential drug candidates in cancer therapy subjected to experimental validation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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