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In silico Molecular Docking, Molecular Dynamic Simulation and ADME Study of New (2-Methyl Benzimidazole-1-yl)-N- Derivatives with Potential Anti-proliferative Activity

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Abstract: Although there has been great progress in the development of anticancer medications, significant hurdles remain, including drug resistance, poor effectiveness, and excessive toxicity, which have all profoundly impacted the daily lives of cancer patients. Therefore, finding highly selective, effective, and non-toxic anticancer drugs constitutes a paramount challenge in contemporary cancer research. We present an in silico evaluation of a new series of 2-methylbenzimidazole derivatives to determine the anti-proliferative effect in the epidermal growth factor receptor (EGFR) active sites. Our six ligands docked with the EGFR crystal structure (protein data bank code: 4HJO) to verify their binding affinity to active sites. One of these ligands showed a high score (75.5) and two ligands had as binding energy as the gold standard drug Erlotinib. The molecular dynamic simulation study proves that ligand 1 had a good Conformational match with the EGFR According to Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) data. After analyzing the ADME study of virtually active compounds, they achieved Lipinski's rules and other pharmacokinetic properties. Lastly, these ligands can function as precursors for the development of novel anti-proliferative drug.

Keywords: Molecular docking, Molecular dynamic, ADME, 2-methyl benzimidazole, EGFR inhibitors, anti-proliferative activity.

1. Introduction

Cancer is a hyperproliferative disorder, which refers to a group of disorders where cells undergo an abnormal transformation, leading to uncontrolled growth, invasion, and dissemination via the lymphatic system or bloodstream. [1-3] Cancer may be a malfunction of cells to initiate apoptosis, a critical mechanism regulating cell proliferation. [3]

The armamentarium against cancer includes surgical excision, radiation therapy, chemotherapy, immunotherapy (which stimulates an immune response resulting in long-term tumor eradication), and molecular targeted therapy (mainly affecting cell Propagation and maintenance, such as tyrosine kinase inhibitors, interferons, and lymphokines). [4, 5]

After cardiovascular disease, cancer is the second cause of mortality worldwide [6]. About 9.6 million people worldwide lose their lives each year due to cancer [7]. Although there has been great progress in the development of anticancer medications, significant hurdles remain, including drug resistance, poor effectiveness, and excessive toxicity, which have all profoundly impacted the daily lives of cancer patients [8]. Therefore, finding highly selective, effective, and non-toxic anticancer drugs constitutes a paramount challenge in contemporary cancer research [9].

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The lack of molecular-level selectivity in the mechanism of action of traditional cancer chemotherapeutic drugs results in their impact on both healthy and cancerous tissues, which ultimately contributes to the development of drug resistance in cancer [10]. Recent encouraging strategies offer a more promising approach: targeted drug therapy, this method aims to overcome the limitations of traditional chemotherapy by honing in on specific cancer-associated molecules, aiming to reduce adverse effects, enhance patient tolerability, and promote the modulation of the host immune system for improved homeostasis. [11]

Targeted anticancer therapies set themselves apart from conventional chemotherapy by their meticulous selection of targets. Instead of a broad attack, they meticulously focus on genes, tissue environments, or proteins specific to cancer that are essential for the tumorigenesis of cancer cells and are excessively expressed in cancerous tissues [12]. Several promising examples of these are tyrosine kinases [13], prostate-specific membrane antigen (PSMA) [14], carbonic anhydrase IX [15], biotin receptor [16], G-protein-coupled receptor 87 in pancreatic cancer [17], growth factor receptor in breast cancer [18], and folate receptor [19]

Protein Tyrosine Kinases (TKs) are a group of enzymes that utilize ATP to phosphorylate tyrosine molecules. They play an essential role as mediators in cascade signaling [20], as shown in Figure (1). [21]

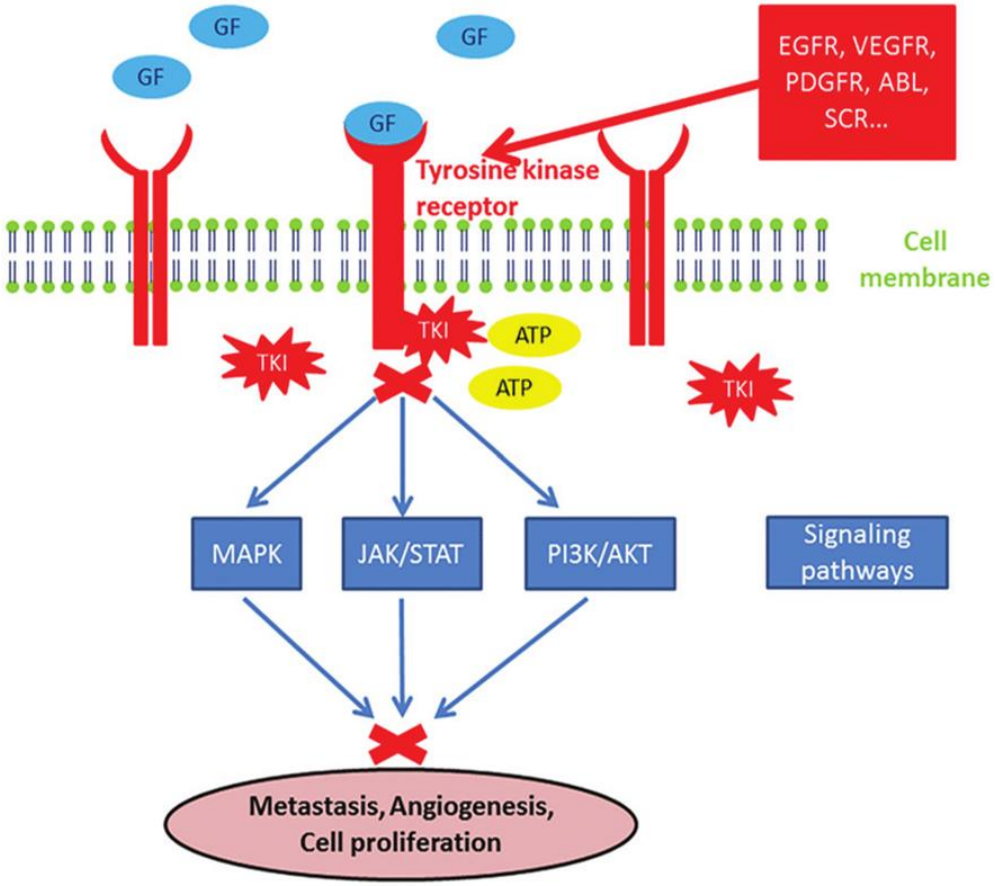


Figure 1. Schematic representation of tyrosine kinase mode of action. [21]

Tyrosine kinases play a pivotal role in the molecular pathogenesis of cancer, and they have lately become viable targets for novel anticancer medicines, resulting in the approval of many of these treatments. The sequencing endeavor of the Human Genome Project has greatly increased the quantity and complexity of tyrosine kinases, offering new opportunities for the development of drugs. Present understanding of the molecular causes of cancer suggests that many tyrosine kinases, especially receptor tyrosine kinases, are situated upstream or downstream of pharmacologically significant tumor suppressors or oncogenes [22]. Epidermal growth factor receptor,

(EGFR) is a member of the ERBB family of receptor tyrosine kinases, emerged as a potential target in the 1980s due to its link with cancer. EGFR overexpression is associated with several hallmarks of cancer, including cell proliferation, inhibition of apoptosis, dissemination, and angiogenesis, all of which contribute to the growth and progression of tumors [23]. Figure 2 illustrates the activation model of EGFR [24].

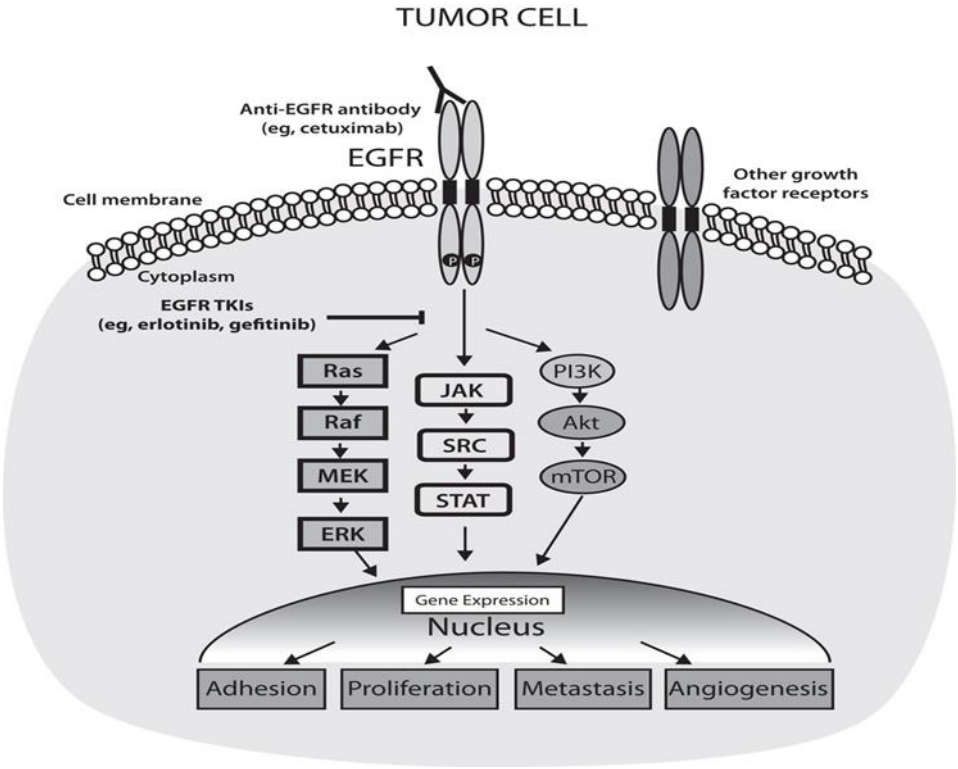


Figure 2. A model of EGFR activation. [23]

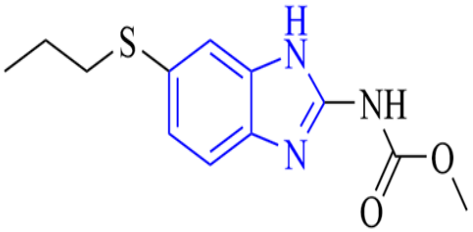
Erlotinib, an FDA-approved drug, exemplifies the success of targeting EGFR in non-small cell lung cancer treatment. [25]

The displacement of adenosine triphosphate (ATP) effectively inhibits the autophosphorylation of the EGFR and subsequent signaling cascades. Due to the unfavorable prognostic value associated with the overexpression of EGFR in these cancers, targeting EGFR has emerged as a promising approach for anticancer therapy. [26&27]

Biological targets can quickly generate hydrogen bonds with nitrogen. Nitrogen heterocycle derivatives are a rich source of potential therapeutic medications in medicinal chemistry. [28]

Anti-cancer, anti-HIV, anti-malarial, anti-tuberculosis, anti-inflammatory, and other therapeutic effects are only some of the many that can be attained through the use of nitrogen-containing heterocyclic compounds. [29]

The field of benzimidazole-based chemistry gained significant momentum in the 1950s following the detection of 5, 6-dimethyl-1-(α-D-ribofuranosyl) benzimidazole (a crucial component of the vitamin B12 molecule). [30]



Compound 1

Figure 3. albendazole

Benzimidazole is considered a purine-based nucleic acid isostere and is recognized as a preferred lead nucleus extensively utilized in developing various biologically active compounds. [31] And demonstrate a diverse array of pharmacological properties, such as antibacterial [32], antifungal

[33], analgesic [34], cardiovascular [35], and anticancer activity [36–38]. They demonstrate potent cytotoxicity against numerous cancer cell lines. [39] Albendazole (compund 1), a commonly employed antiparasitic medication, exhibits significant anti-proliferative efficacy against colorectal cancer (CRC) and hepatocellular carcinoma (HCC).[40&41]

Recent years have seen a surge in the development of benzimidazole-derived anticancer medicines stems from their diverse biological activities and favorable safety profiles. Due to their structure similarity to purine and its derivation from vitamin B12, benzimidazole is a preferred substructure for interacting with biopolymers, leading to a compatible system for biologically active molecules. [42&43]

Binimetinib (2), bendamustine (3), selumetinib (4), pracinostat (5), and galaterone (6) are examples of benzimidazole-based molecules with clinical approval. [44]

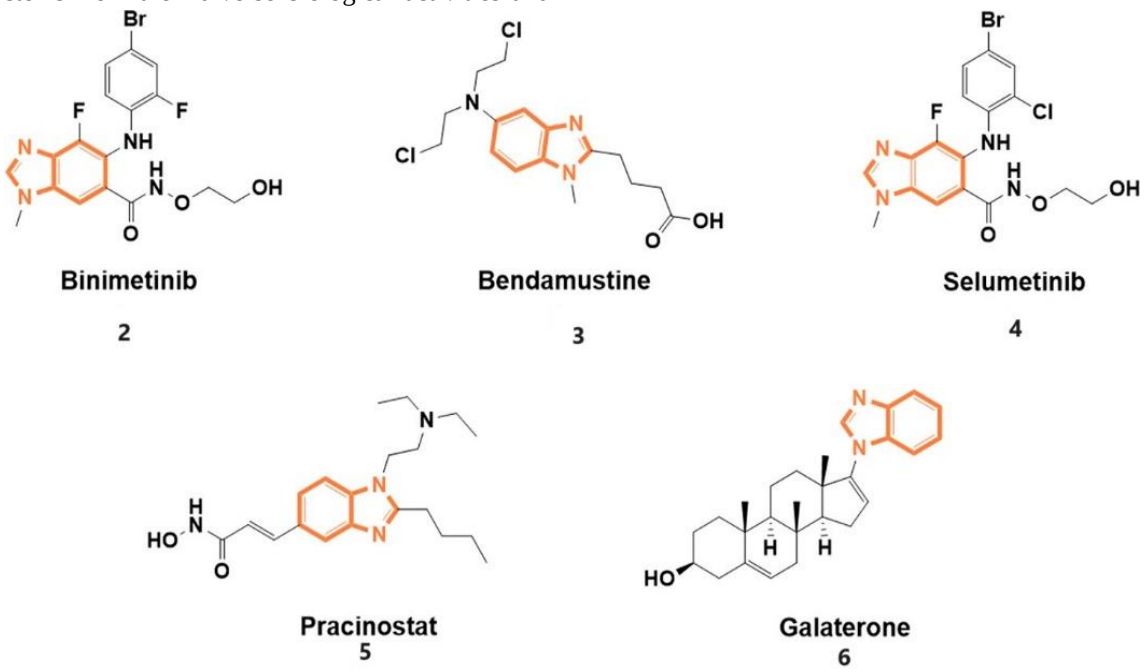


Figure 4. Benzimidazole-based clinically approved anticancer drugs

The presented evidence highlights the remarkable potential of benzimidazole and its derivatives as a prominent scaffold for the development of next-generation anticancer drugs. Their unique structural properties, diverse biological activities, and promising safety profile position them as a valuable tool in the fight against cancer.

The study seeks to accomplish the following:

- Utilize computational docking techniques to elucidate the binding affinity of newly designed benzimidazole analogues towards their target proteins.
- Employ PLP scoring function to evaluate the binding affinity of six newly designed ligands towards the epidermal growth factor receptor (EGFR), to decipher the ligand-receptor interaction mechanism.
- Leverage in silico ADME prediction to assess the drug-like nature and pharmacokinetic behavior of the designed compounds.
- Apply Molecular Dynamics (MD) simulations that offer a powerful tool to investigate the behavior and interactions of molecules at the atomic level. Which provides valuable insights for understanding, predicting properties, and validating experimental data.

2. Computational Method

2.1. protein receptor and ligand preparation

Crystal structure of epidermal growth factor receptor complexes with erlotinib acquired from the Protein Data Bank (PDB) (PDB code: 4HJO). To attain amino acid residues, the right ionization

tautomeric states in the structures of target proteins, water molecules were removed except those included in the active site, and hydrogen atoms were added.

The structures of the ligand molecules are drawn using ChemDraw (version 22.0.022).[45]

To reduce the six selected ligand molecules' energy, a molecular mechanic force field was implemented in Chem3D (version 22.0.022).

2.2. Docking procedures

The molecular docking method made use of the Cambridge Crystallographic Data Centre's (CCDC) Genetic Optimization for Ligand Docking (GOLD) program.[46&47] ChemDraw, another piece of Cambridge-created software, was also used. For this examination, we used v2022.3.0. The receptor was prepared for docking and the images were captured using the Hermes visualizer (version 2022.3.0, Cambridge, England) as part of the GOLD suite. The GOLD docking binding site consists of all protein residues within 10A˚of the reference ligands in the downloaded protein structure complexes.

ChemBioOffice, (version 22.0.022), was used to display the chemical structures of the ligands.

To perform ensemble docking, multiple distinct epidermal growth factor receptor proteins (1M17, 4HJO, and 2ITY) were downloaded from the PDB database. [48]

Thus, the EGFR protein crystal structure 4HJO was selected since it shows an interaction with erlotinib. The ChemPLP fitness was employed as the scoring function in this study, which was designed using the Chemscore kinase. The final posture number was not altered; 10 was kept as the default; and the highest-ranked solution was designated the default. To learn how the ligands we designed interacted with the EGFR protein's amino acid residues, we analyzed docking data. In this study, we considered the effects of docking position, binding free energy, and binding mode.

2.3. Molecular dynamic simulations

MDS was performed for the derivative with the best docking score using the Desmond modules of the Schrodinger 2023 with the OPLS4 force field. To create a charge-neutral system for the protein-ligand complex, sodium ions were added, and 0.15 M sodium chloride NaCl was included to mimic the natural system. Utilizing the TIP3P solvent model, the system was produced. The simulation was run for 50 ns, with recording intervals of 50 ps for the trajectory. The NPT ensemble class was utilized, and the system energy was set to 1.2. The simulation was set to operate at 1.01325 bar and 300 k. To create the simulation interaction diagram, the simulated system was evaluated after it was relaxed. [49&50]

2.4. ADME procedure

The pharmacokinetic profile of the virtually active compounds, including their BBB (blood-brain barrier) permeability, affinity for P-gp, and bioavailability, was evaluated using the Swiss ADME server. To identify therapeutic candidates that exhibit optimal safety and promise, a crucial step involves the exclusion of compounds possessing inadequate ADME (absorption, distribution, metabolism, and excretion) properties, which are prone to failure during subsequent stages of drug development.

The ligands were initially created using the ChemDraw software and subsequently exported as SMILE names through the utilization of the SwissADME tool. The polarity and lipophilicity of the compounds were evaluated using the BIOLED-Egg. [51]

3. Results and discussion

Examining the contact interactions between the protein's active binding sites and our compounds allowed us to determine the selectivity, affinity, and binding energies of the ligands for the EGFR through docking. The ranking of the EGFR inhibitory activity of compounds 1-6 and erlotinib was determined based on their PLP fitness. The docking analysis revealed that several amino acid residues, including THR 830, THR 766, MET 769, LEU 694, VAL 702, LYS 721, LEU 820, ALA 719, GLY 772, LEU 834, GLY 695, ASP 831, ILE 720, ASP 766, CYS 773 inside the active site of EGFR engage in interactions with our final ligands through hydrogen bonding and short contacts. The docking result of the inhibitors against the EGFR receptor is shown below in Table 1.

Sex best compounds have been chosen, it was observed that all compounds are binding to EGFR active sites with good binding energies.

As shown in Table (1), compound (I) has the highest PLP fitness of 75.5 and the strongest H-bonding with amino acids. Compounds II and III have as binding energies as the gold standard drug Erlotinib, which contributes to the 73.6,73.4 and 74 fitness values of the PLP respectively.

Other compounds (IV, V & VI) have slightly less binding energies than erlotinib, 71.1, 69.8 & and 65.8 respectively, and binding to the same amino acids that the standard drug erlotinib binds to it and very good results.

As illustrated in table (1) compound (I) forms two H-bonds, both of them through carbonyl of amide group with THR 830 and THR 766 by water molecule and hydrophobic interactions with, THR 830, MET 769, THR 766, LEU 694, VAL 702 & LYS 721(as shown in figure 7) and provide PLP value (75.5).

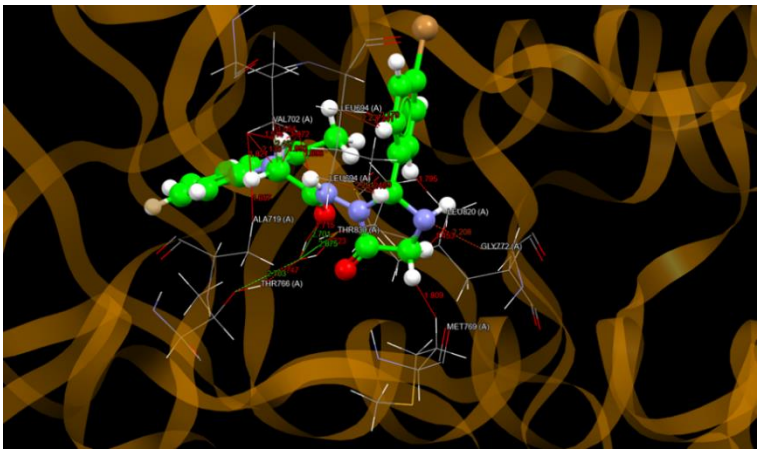


Figure 7. 3D representations of interaction between new 2-methyl benzimidazole derivative 1 and amino acids in EGFR active sites

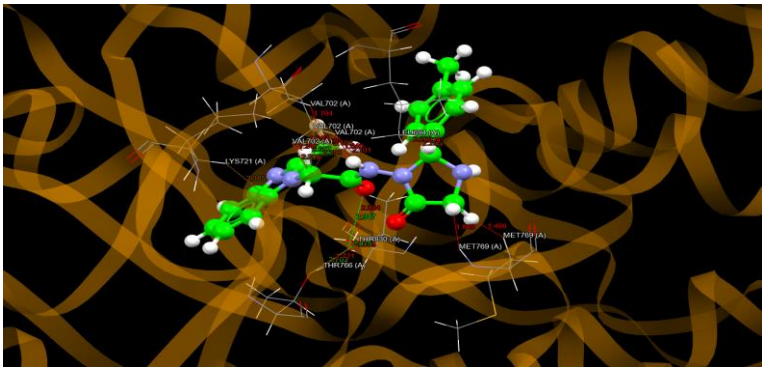


Figure 8. 3D representations of interaction between new 2-methyl benzimidazole derivative 2 and amino acids in EGFR active sites

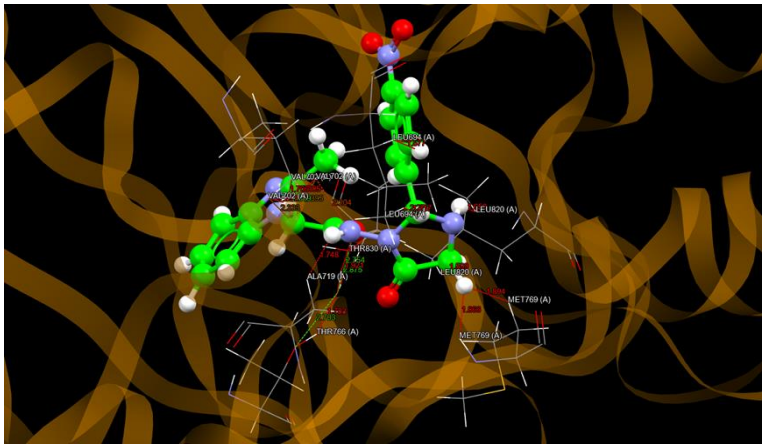


Figure 9. 3D representations of interaction between new 2-methyl benzimidazole derivative 3 and amino acids in EGFR active sites

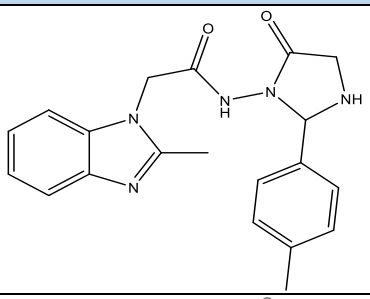
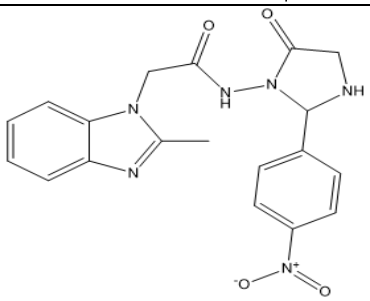
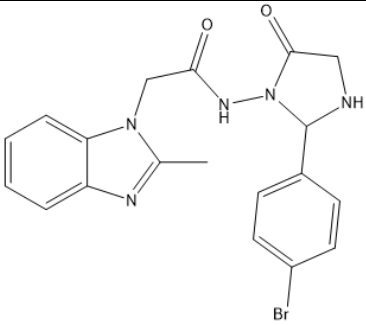
Compound (II) forms two H-bond through carbonyl of amide group with THR830 and THR 766 by water molecule and hydrophobic interactions with LEU 694, LEU 820, MET 769, THR 830, THR 766, VAL 702 & ALA 719 (as shown in figure 8)and gives PLP value (73.6).

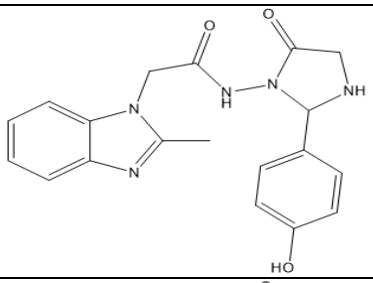
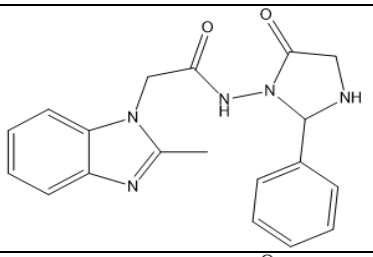
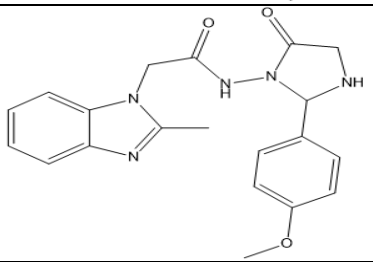
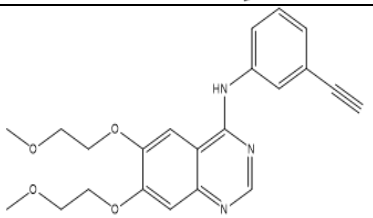
Compounds (III) form two H-bonds through carbonyl of amide group with THR830 and THR 766 by water molecule and hydrophobic interactions with LEU 820, GLY 772, LEU 694, VAL 702, MET 769, ALA 719, THR 766 & THR 830 (as shown in figure 9) and gives PLP value (73.4).

These amino acids are present in the epidermal growth factor receptor's active sites binding of erlotinib.

The previously mentioned interactions have enhanced the capabilities of newly synthesized compounds and designated them as anti-proliferative agents with greater effectiveness and binding affinity.

Table 1. The epidermal growth factor receptor active site docking score for several 2-methylbenzimidazole derivatives.

Number of 2-methyl Benzimidazole derivatives	Structure	Docking score	Binding interactions
1		**75.5**	**H-interactions** THR 830, THR 766 **Hydrophobic interactions** MET 769, THR 830, THR 766, LEU 694, VAL 702, LYS 721
2		**73.6**	**H-interactions** THR 830, THR 766 **Hydrophobic interactions** LEU 694, LEU 820, MET 769, THR 830, THR 766, VAL 702, ALA 719
3		**73.4**	**H-interactions** THR 830, THR 766 **Hydrophobic interactions** LEU 820, GLY 772, MET 769, LEU 694, VAL 702, ALA 719, THR 830, THR 766.

4		**71.1**	**H-interactions** THR 830, THR 766 **hydrophobic interactions** LEU 694, MET 769, VAL 702, THR 766, THR 830, LEU 834
5		**69.8**	**H-interactions** THR 830, THR 766 **Hydrophobic interactions** VAL 702, LEU 694, GLY 695, GLY 772, LEU 820, THR 830, THR 766
6		**65.8**	**H-interactions** THR 830, THR 766 **Hydrophobic interactions** VAL 702, LEU 820, ASP 831, LYS 721, ILE 720, GLY 695, LEU 694, MET 769, ALA 719, THR 830, THR 766
Erlotinib		**74**	**H-interactions** CYS 773, THR 830, THR 766 **Hydrophobic interactions** ASP 766, CYS 773, GLY 695, VAL 702, THR 766

The compound 1-EGFR complex was subjected to MDS analysis to confirm the interaction. Simulations of molecular dynamics (MD) are a proven method used to understand macromolecular ligand-receptor interactions effectively. Also, one of the strengths of MD modeling is that it can account for the flexibility and movement of proteins. The highest-scoring ligand was subjected to MD simulations to understand the evolution of receptor binding ability over time. The dynamic behavior of ligand 1-EGFR was studied and recorded for 50 ns. The stability of the protein-ligand complex was assessed by studying RMSD and RMSF values.

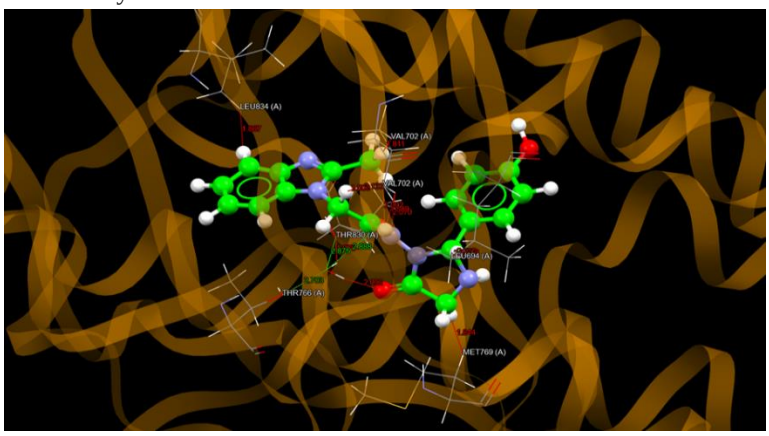


Figure 10. 3D representations of interaction between new 2-methyl benzimidazole derivative 4 and amino acids in EGFR active sites

Analysis of the root-mean-square deviation (RMSD) indicated that the ligand maintained a stable binding pose with the protein complex, exhibiting an average deviation of less than 3.5 angstrom. Notably, the RMSD of the protein remained below 2 angstrom at the bound state, suggesting minimal structural changes upon ligand binding. If the value observed of a ligand is significantly larger than the RMSD of the protein, the ligand has most likely diffused away from its initial binding site, while our results showed that the ligand aligned with the protein and underwent similar conformation variation along the simulation time.

These results indicate that the ligand is quite stable (as shown in Figure 14). Reinforcing the notion of structural stability, RMSF analysis revealed that the majority of protein residues exhibited a root-mean-square fluctuation (RMSF) below 1.0 Å. This minimal deviation from the average position signifies a high degree of rigidity within the protein structure (as shown in Figure 15). It is possible to describe variations in the locations of the ligand atoms using the Ligand Root Mean Square Fluctuation (L-RMSF). Compound 1's RMSF value demonstrates how well it fits with the protein, Compound 1 RMSF of less than 1 with some fluctuation at groups 21, 22, and 23,24,25,27 (as shown in figure 15) demonstrates a stable compound.

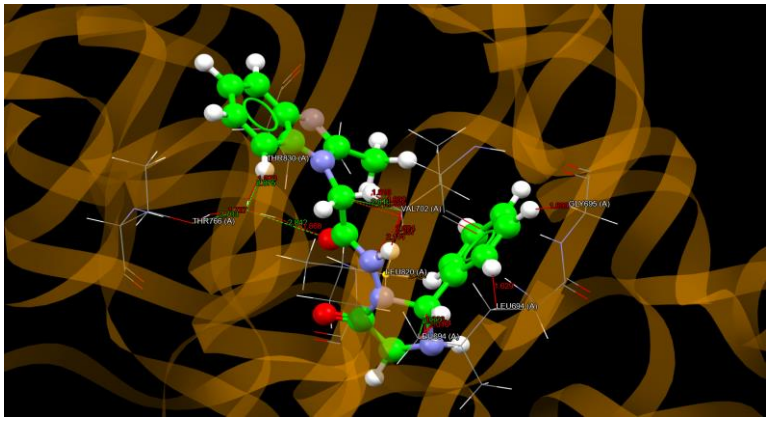


Figure 11. 3D representations of interaction between new 2-methyl benzimidazole derivative 5 and amino acids in EGFR active sites.

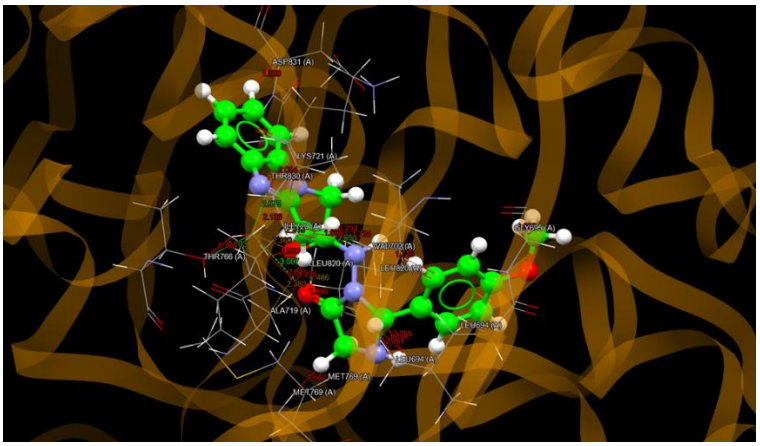


Figure 12. 3D representations of interaction between new 2-methyl benzimidazole derivative 6 and amino acids in EGFR active sites

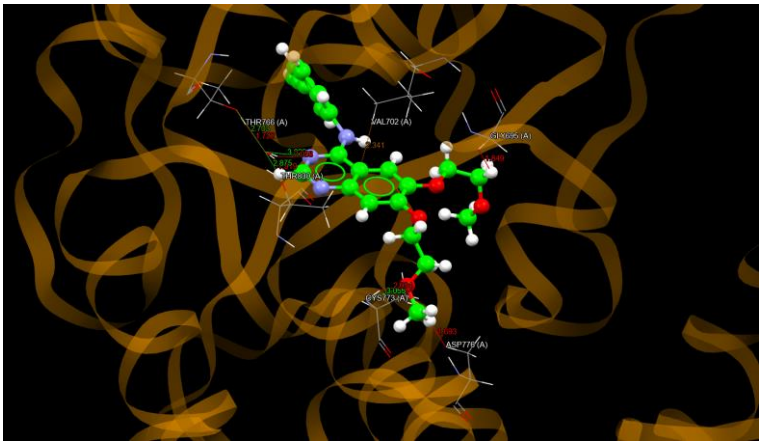


Figure 13. 3D representations of interaction between standard drug erlotinib and amino acids in EGFR active sites (Green dashes represent H-interactions & and red dashes represent short contacts)

Assessment of the ADME process (absorption, distribution, metabolism, and elimination) should begin at an early stage of the research process, a drug may not be considered for further development if it has unfavorable ADME attributes. To assess the potential of our compounds, we employed the SwissADME online tool to evaluate their properties.

Figure 16 illustrates a representation of BOILED-Egg. It has been demonstrated that compounds 1–6 (blue dots) are poorly able to cross the BBB and are considerably absorbed passively from the gastrointestinal tract.

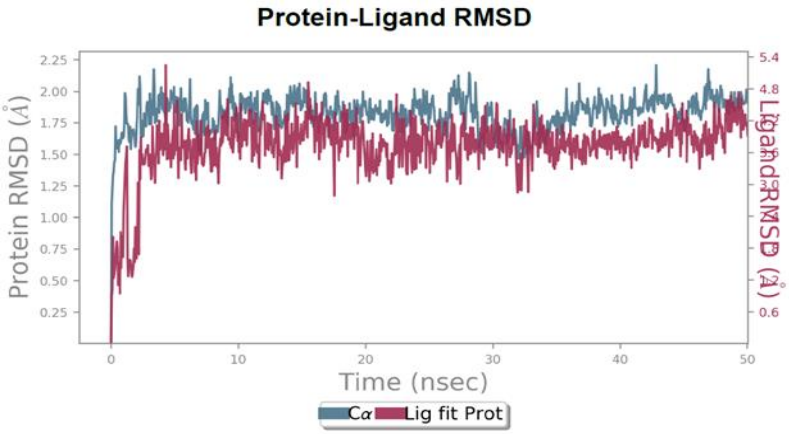


Figure 14. Ligand 1 –EGFR complex RMSD.

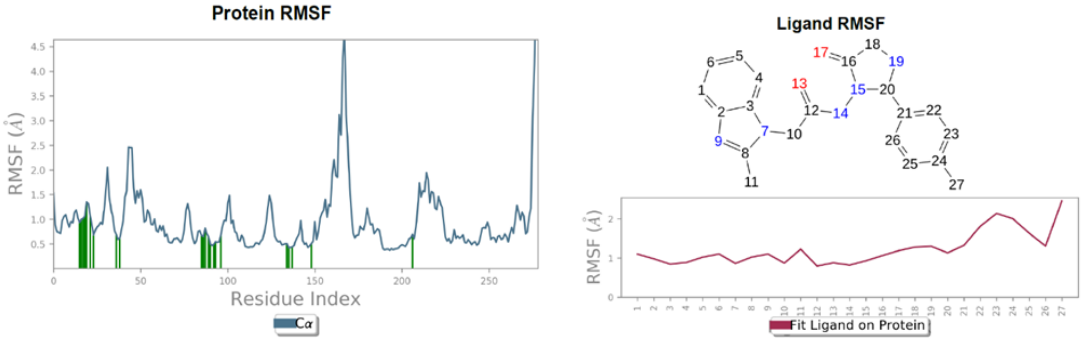


Figure 15. Ligand 1 and EGF receptor RMSF.

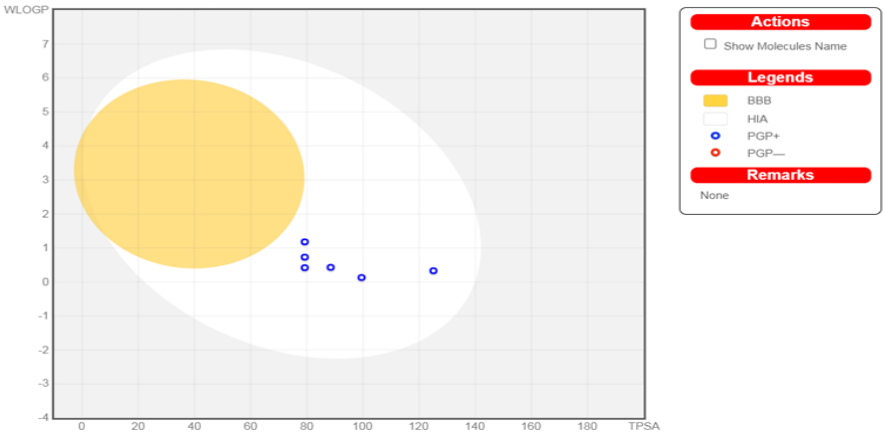


Figure 16. BOILED-Egg for new 2-methyl benzimidazole derivatives (1-6), (blue dots). Molecules within white ovules can be absorbed through GIT. Blue dots are assigned to molecules that are considered P-glycoprotein substrates (PGP+), which can export from the central nervous system.

Medications intended for oral administration must comply with Lipinski's rule of 5, which states that they should have a molecular weight of less than 500, a partition coefficient (o/w) of less than 5, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors. [52]

Table 2 demonstrates that all of our compounds followed Lipinski's rules.

Furthermore, the drug must possess a polar surface area of less than 140 Å, as this is an essential property directly linked to its bioavailability. There exists an inverse relationship between the PSA levels and the oral bioavailability of the medicine, to enhance the oral bioavailability, all substances must undergo passive absorption, which is facilitated by having a topological polar surface area (TPSA) of less than 140 Å and as we show in table 3 all our compounds have TPSA less than 140 Å.

Table 2. ADME study results for different new 2-methyl benzimidazole derivatives

Compounds	TPSA	Water solubility	G.I Absorption	BBB Permeability	Lipinski	Veber
1	79.26 Å²	Soluble	High	No	yes	yes
2	125.08 Å²	Soluble	High	No	yes	yes
3	79.26 Å²	Moderately Soluble	High	No	yes	yes
4	99.49 Å²	Soluble	High	No	Yes	Yes
5	79.26 Å²	Soluble	High	No	Yes	Yes
6	88.49 Å²	Soluble	High	No	Yes	Yes

4. Conclusions

One of the most effective drug discovery methods is Molecular docking. In an effort to develop improved EGFR inhibitors, this study utilized a structure-based computational drug design approach. This strategy aimed to identify selective candidates that could surpass the limitations associated with current therapies.

Consequently, these compounds show increased anti-proliferative activity and binding affinity.

Compounds have good pharmacokinetic and physicochemical characteristics and also match Lipinski's principles. The molecular dynamics simulations indicated that the stability of the Ligand 1-EGFR complex was maintained, with crucial protein-ligand interactions preserved throughout the simulation. Additionally, the results showed that Ligand 1 exhibited acceptable RMSD and RMSF values, along with effective interaction with EGFR enzymes. Lastly, these ligands can function as precursors for the development of novel anti-proliferative agents. As a result, to determine their effectiveness, side effects, and toxicity profile, all proposed compounds must go through extensive in vivo and in vitro studies.

Acknowledgments
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Abbreviation
ADME: absorption, distribution, metabolism, excretion
ATP: adenosine triphosphate
BBB: blood-brain barrier
CCDC: the Cambridge Crystallographic Data Centre
CRC: colorectal cancer
EGFR: Epidermal growth factor receptor
FDA: food and drug administration
G.I: gastro-intestinal
GOLD: Genetic Optimization for Ligand Docking
HCC: hepatocellular carcinoma
HIV: human immunodeficiency viruses
MD: molecular dynamic
Ns: nanosecond
PDB: protein data bank
P-gp: p-glycoprotein
PLP: piecewise linear potential scoring
RMSD: Root Mean Square deviation
RMSF: Root Mean Square Fluctuation
RTK: receptor tyrosine kinase
TPSA: a topological polar surface area

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