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In-silico molecular docking, ADME study, and molecular dynamic simulation of new azetidin-2-one derivatives with antiproliferative activity

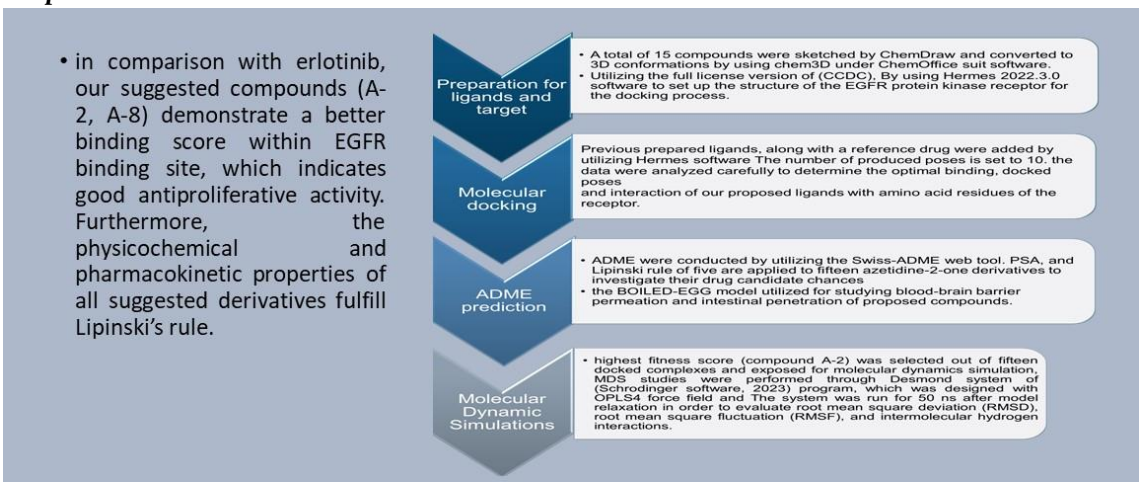
Aya Ahmed[1], **Monther Faisal**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mustansiriyah University, Baghdad 10011, Iraq.

Abstract: Over the past two decades, protein kinase has been a heavily studied target in the development of new anti-proliferative medications. Heterocyclic systems have been identified as the preferred scaffold because of their wide range of biological properties. In this research, the objective was to design and develop fifteen novel azetidin-2-one derivatives and assess their cytotoxic potential as inhibitors of the epidermal growth factor receptor, which is considered one of the key factors influencing cell growth and proliferation. The crystal structure of inactive EGFR tyrosine kinase domain ligand erlotinib from protein data bank was retrieved in order to be docked with our proposed azetidine-2-one derivatives to evaluate their activity as anti-proliferative agents. In this article, an in-silico molecular docking approach proposes that these azetidine-2-one derivatives have satisfactory binding contact with the erlotinib binding site. Although, three compounds have been identified as the most powerful as they have PLP fitness scores of (77.79, 76.68 and 71.46), respectively, while the reference ligand's fitness score was (71.94). Additionally, all of our derivatives have satisfied the Swiss-ADME parameters, indicating that they may be orally active compounds. In conclusion, two compounds (A-2 and A-8) have better PLP fitness, and one (A-14) has a comparable score in comparison to the reference ligand, at the active site of epidermal growth factor receptor. indicating that the novel azetidine-2-one derivatives have shown interesting results and could be used as model compounds to create novel anti-proliferative drugs. However, more pharmacological evaluation is needed.

Keywords: Azetidin-2-one, imidazole, EGFR inhibitors, anti-proliferative, molecular dynamic simulation

Graphical abstract



[1] Corresponding Authors

e-mail: aya_ahmed@uomustansiriyah.edu.iq

Highlight

- *New Drug A-2 shows fitness score higher than reference drug.*
- *New drug may show promising effect on cancer cell proliferation.*
- *All azetidine-2-one derivatives fulfils Lipinski rules.*
- *The RMSD of compound A-2 was assessed and showed a deviation of approximately 1Å, which indicates a perfectly stable ligand*

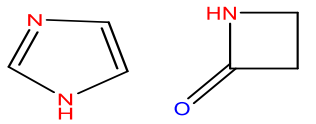
1. Introduction

Internationally, cancer is the second-highest cause of death. The term "cancer" refers to a group of diseases that emerge when DNA damage accumulates in cells, leading to uncontrolled growth and cell development. Cancer cells have the ability to spread throughout the body and are invisible to the immune system. This implies that the therapy method should be selective for cancer cells more than normal cells. For this reason, investigations on the production of novel inhibitors for cancer have attracted attention [1]. Anticancer medications have multiple targets. These include blocking particular receptors and interfering with certain proteins or enzymes, which can destroy cancer cells. The most significant targets are as follows: kinase inhibition [2], DNA-intercalation [3,4], apoptosis stimulation [5-7] and tubulin polymerization inhibition [8,9]. In the last 20 years, protein kinase has been an intensively studied target for the production of novel anti-tumor medications [10]. The epidermal growth factor receptor (EGFR) which is transmembrane glycoprotein belongs to ErbB family of tyrosine kinase receptors. It serves as main target site for medications like gefitinib or erlotinib. The receptor tyrosine kinase is auto phosphorylated when ligands bind to EGFR, which further activates the associated signal transduction pathways. However, blocking EGFR prevents transduction and causes cancer cell death [11].

In the field of drug discovery, heterocyclic systems have been recognized as important scaffolds due to their broad range of biological and pharmacological properties [12]. Among these are the earlist acridine derivatives that have garnered significant attention since the 19th century for their potential pharmacological activity and industrial applications [13]. Additionally, azetidine-2-ones, commonly referred to as β-lactams, are widely utilized as active moieties in medication design due to their safety profile [14-16].

Furthermore, β-lactams possess a variety of biochemical activities, including antibacterial, antifungal, analgesic, anti-inflammatory, antitumor, and antiviral activity. The anti-bacterial activity of the four-membered heterocyclic ring is widely known [17-20]. Hermann Staudinger produced the first synthetic β-lactam in 1907 using [2+2] cycloaddition of ketenes with imines (Staudinger synthesis) [21].

However, cancer treatment has recently focused on heterocyclic compounds, notably those that have a nitrogen atom. Between these, imidazole, a five-membered azole ring as shown in figure (1), is widely used and has the opportunity to resolve the problems of commercial medications [22].



1*H*-imidazole azetidin-2-one

Figure1. structure of azetidinone and imidazole.

In order to investigate the anticancer activity, Yusuf Ozkay *et. al.*, in 2004 found a group of novel derivatives of imidazole epiperazine and imidazole-(Benz) azole. Cisplatin was utilized as a reference medication. Anticancer activity results indicated that the new compounds had the most anticancer activity, as shown in figure (2), [23].

According to recent research, theoretical calculations assist in the development of more potent and active compounds, which are important for drug discovery at various stages, from synthesis and characterization to activity comparison [24]. Furthermore, theoretical techniques have advanced significantly over the past ten years as a result of developments in technology to fully understand the interactions between compounds and protein active sites [25].

Commercially available chemotherapeutic drugs have shown potent anticancer activity, however, several challenges need to be overcome, such as undesirable side effects, drug resistance, and toxicity on normal cells as a result of their lack of selectivity. Therefore, in order to find new therapies

with fewer systemic side effects, a new rationale for antitumor drugs should be looked into. In particular, azetidinone, which attracted scientists' interest.

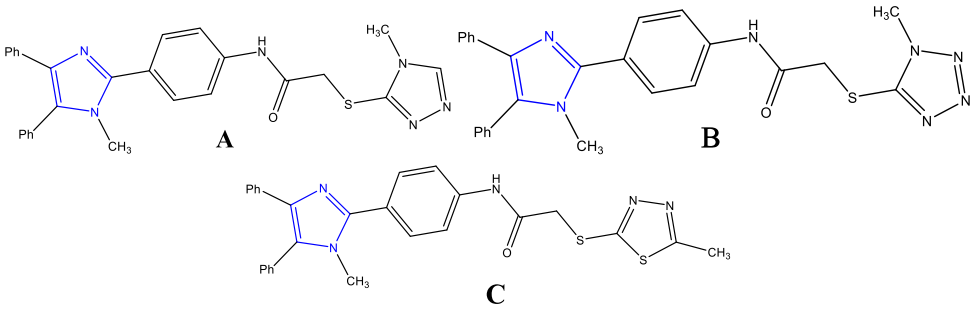


Figure 2. Imidazole derivatives with potent anticancer activity.

2. Computational Method

In this study, our proposed compounds were examined using the docking protocol, which is a method utilized to calculate the preferred orientation of a ligand when bound to a protein or enzyme to form a stable complex. The three-dimensional crystal structure of the inactive EGFR tyrosine kinase receptor protein complexed with Erlotinib (PDB code: 4HJO) was selected for this study figure (3).

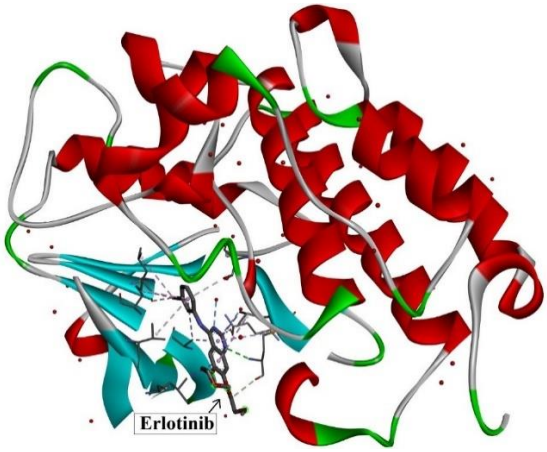


Figure 3. The three-dimensional crystal structure of erlotinib bound to inactive EGFR (PDB code: 4HJO), inside active site of chain A. erlotinib is represented as stick model.

2.1. Preparation for ligands

A set of fifteen 3-chloroazetidin-2-one (β-lactam) derivatives was provided according to a literature review. The new study focuses on activity. We offered our work and supported our results with a docking study after confirmation that the proposed compounds had never been synthesized. A total of 15 compounds were sketched by ChemDraw Professional and converted to 3D conformations by using chem3D under ChemOffice suit software (ChemOffice, 2020 v.20.0.). From this point, chem3D with the MM2 force field was used to optimize the molecule's lowest energy conformation, saving it in SYBYL2(mol2) format.

2.2. Preparation of target

Various different X-ray crystal structures were available on the Protein Data Bank server. We used the structure of the EGFR protein along with erlotinib (PDB code: 4HJO) to dock the newly sketched ligands. Utilizing the full license version of Genetic Optimization for Docking Cambridge Crystallographic Data Centre (CCDC), running on Windows 11 operating system. By using Hermes 2022.3.0 software from CCDC Gold suit to set up the structure of the EGFR protein kinase receptor for the docking process by eliminating additional chains while maintaining chain A, and all water molecules with a reference ligand (erlotinib) were extracted from the active site of the receptor. Finally, hydrogen atoms were added to the active site.

2.3. Molecular docking

After preparing the proposed ligands and protein as mentioned earlier, we added these ligands, along with a reference drug and the required water molecule needed for hydrogen bonding that was extracted earlier, to the protein by utilizing Hermes software. The number of produced poses is set to 10, according to docking performance. Gold determines an individual's fitness based on the ChemPLP fitness score, stronger attachment between the protein kinase receptor and our suggested ligands is indicated by a high fitness score. Finally, the data was saved. The recorded

data were analyzed carefully to determine the optimal binding, docked poses, free energy binding, and our proposed ligands' contacts with the receptor protein's amino acid residues.

2.4. ADME prediction

The in-silico computational techniques employed to minimize the number of experimental drug trials and enhance the chances of success. Pharmacokinetic properties, including absorption, distribution, metabolism, and excretion, in addition to other parameters such as blood-brain barrier penetration, affinity to p-glycoprotein, toxicity of the proposed ligands, and drug similarity properties, were conducted by utilizing the Swiss-ADME web tool. Swiss ADME server play a major role in computer aided drug design, which determines the most potent compound of all proposed ligands with the best drug-likeness properties prior to their application as pharmaceutical aids. A crucial characteristic associated with bioavailability is polar surface area PSA<140A^{o}, this suggests a drug with a lower PSA possesses a higher oral bioavailability, in addition to the Lipinski rule of five. In this research, the previously mentioned parameters are applied to fifteen azetidine-2-one derivatives to investigate their drug candidate chances, and the final data results are retrieved from Swiss-ADME, table (2).
Additionally, another feature of the Swiss-ADME tool is the BOILED-EGG graphical model utilized for studying blood-brain barrier permeation and intestinal penetration of proposed compounds. Besides that, it possesses the most precise prediction of the novel analogue's polarity and lipophilicity.

2.5. Molecular dynamics simulations

Molecular dynamics simulations are now the preferred technique that is applied effectively to understand the evolution of ligand-receptor binding [26]. Also, unlike the more static molecular docking method, the MD technique does not ignore the fact that proteins change over time. We selected the highest fitness score (compound A-2) out of fifteen docked complexes that were exposed for molecular dynamics simulation based on the total number of hydrogen bonds and its affinity for bindings. MDS studies were performed through Desmond system of (Schrodinger software, 2023) program, which was designed with OPLS4 force field. System building included the addition of counter ions for the system's neutralisation, and 0.15 M NaCl was Provided in order to imitate the physiological conditions. The solvation of the protein-ligand complex was done by utilizing TIP3P water model after eliminating water molecules from the crystal structure of the complex. System creation was followed by MDS, which was inspected for 50 ns by keeping a 50 ps recording interval of energy simulation. The preprocessed system was subjected to gradual heating from 0 to 300 K at 1.013 bar pressure (NPT condition). The system was run for 50 ns after model relaxation in order to evaluate root mean square deviation (RMSD), root mean square fluctuation (RMSF), and intermolecular hydrogen interactions. The obtained data was recorded in order to examine the novel compound's stability as well as the protein's conformational behavior.

3. Results and discussion

Compounds are chosen by using computer programs that analyze how properly they bind to receptor proteins [27]. Gold determines the hydrogen bonds, as well as short contact and the bond distances, PLP fitness score of all proposed ligands binding within active site of EGFR ranges between 60 and 77, as listed in table (1), while interactions of reference drug erlotinib is (71.94) with amino acids THR766 and THR830 through HOH1104 water molecule by hydrogen bonding, in addition to hydrophobic interactions through VAL702 and GLY695 amino acids within EGFR protein, as shown in figure (4).
The docking result reveals that VAL702, GLY695, GLY772, CYS773, THR766, THR830, MET769, MET742, PHE699, SER696, ASP831, LYS721, LEU764, LEU768, LEU694, LEU820, and LEU834 amino acid residues of EGFR active site bind by hydrogen bonds and short contacts with our compounds. In particular, compound (A-2) tops the chart. Among the ligands, compounds (A-2, A-8, and A-14) have a favorable binding affinity compared to the standard drug erlotinib, as shown in figure (5).
The interactions listed above have improved the abilities of the recently designed compounds and candidates the best scored ligand for further MDS study.

BOILED-egg representation of two highest scoring azetidine-2-one derivatives is provided in figure (6) which shows that both (A-2, A-8) have BBB penetration ability. Molecule A-8 (blue dot) could be exported by p-glycoprotein from the central nervous system, On the other hand, molecule A-2 (red dot) is not considered to be a p-glycoprotein substrate.

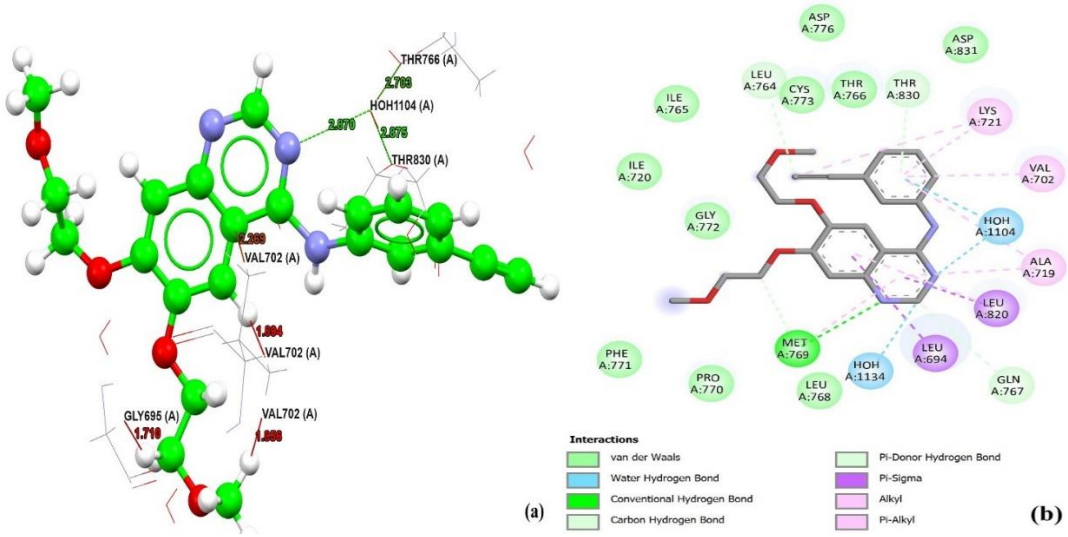
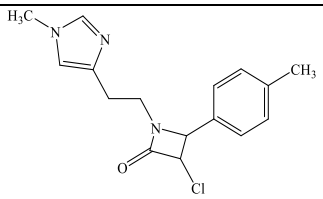
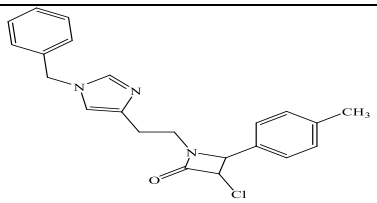
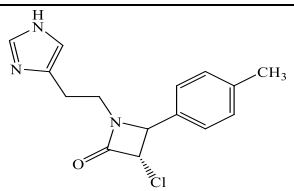
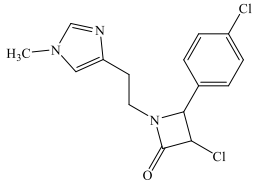
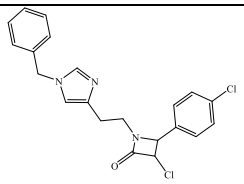
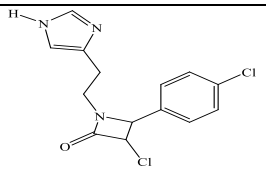
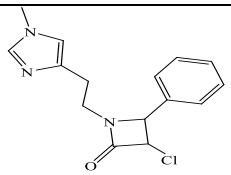
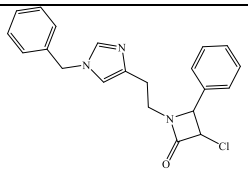
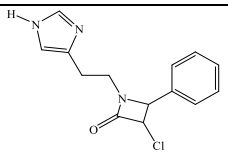
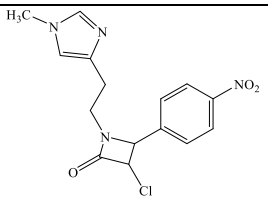
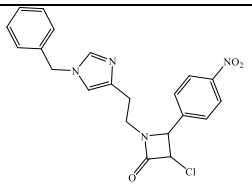
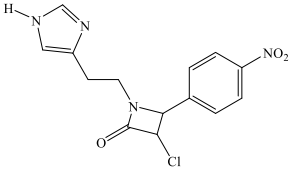
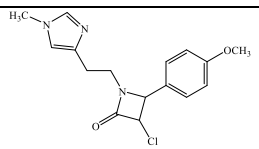
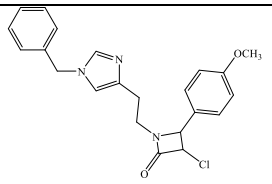
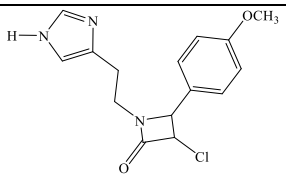


Figure 4. a) 3D docking pose showing interactions of reference drug erlotinib within amino acids of EGFR active site retrieved from PDB docked by using CCDC GOLD suit, b) 2D simple representation of EGFR receptor active site equipped with erlotinib by utilizing Discovery Studio 2021 software, (PDB code: 4HJO).

Table 1: docking results for fifteen Azetidin-2-one derivatives within active site of epidermal growth factor protein receptor.

No. of compound	Structure	PLP fitness score	H-bond interactions	Short contact interactions
A-1		60.86	THR830+THR766 by HOH1104	THR830 +VAL702 +MET769 +GLY772
A-2		77.79	THR830 &THR766 by HOH1104	MET769 +GLY695 +VAL702 +LEU764 +MET742
A-3		57.00	THR766&THR830 by HOH1104	LYS721 +VAL702 +LEU768

A-4		62.00	THR766 + THR830 by HOH1104	LYS721 +THR830 +VAL702
A-5		69.79	ASP831	GLY695 +LEU694 +LEU764 +VAL702 +LEU820 +LYS721
A-6		57.27	THR766+TH R830 by HOH1104	THR830+MET769 +VAL702
A-7		65.73	THR830 +THR766 by HOH1104	LEU834 +THR830 +VAL702 +GLY772 +MET769
A-8		76.68	No Hydrogen bonding	LEU764 +VAL702 +MET769
A-9		56.14	THR830&TH R766 by HOH1104	MET769 +GLY772 +VAL702
A-10		67.18	THR830 +MET769+T HR766 by HOH1104	LYS721 +VAL702 +THR830
A-11		70.44	MET769 + THR830&TH R766 by HOH1104	MET769 +LEU694 +VAL702 +SER696
A-12		58.11	ASP831 +MET769 +THR830&T HR766 by HOH1104	LYS721 +VAL702
A-13		65.51	THR830 +THR766 by HOH1104	LEU834 +THR830 +VAL702 +LEU694 +MET769

A-14		71.46	MET769	MET769+LEU820 +LEU694 +VAL702 +GLY695 +PHE699
A-15		58.73	MET769	VAL702 + MET769 +LEU820 + LYS721 + THR766

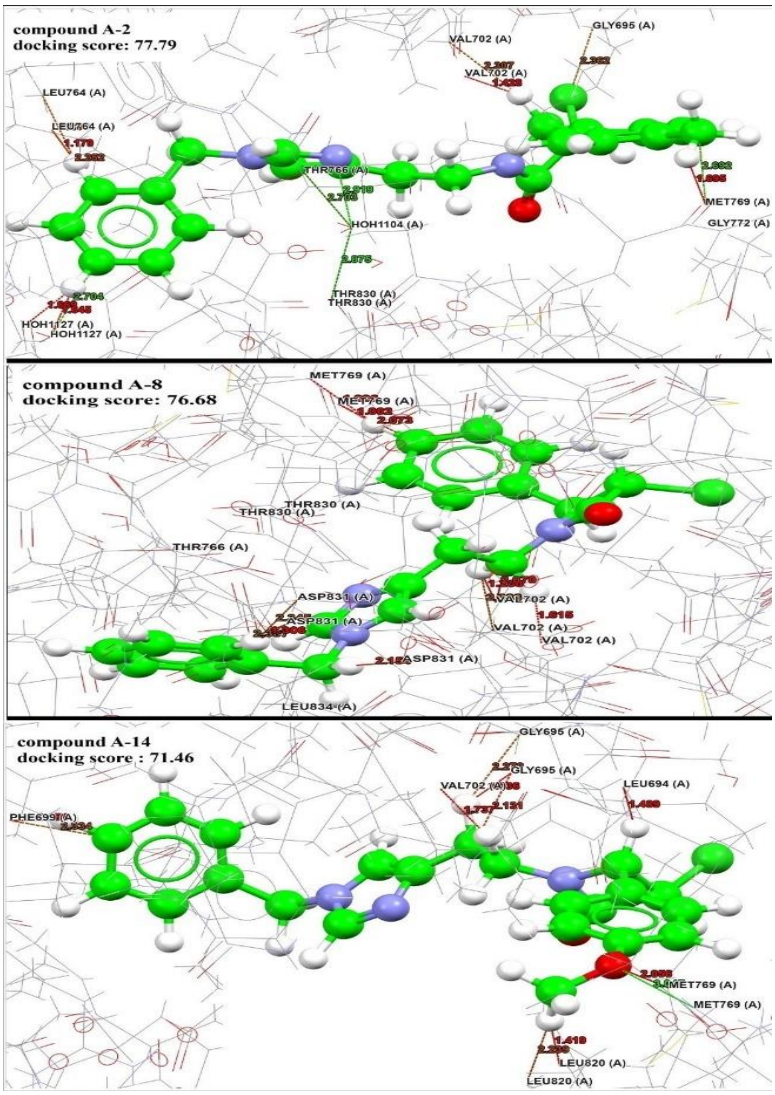


Figure 5. Chemical interactions of the three highest docking score lead molecules within the active site of EGFR.

A crucial measure of the drug transport process is TPSA, or topological polar surface area, which describes the bioavailability as well as intestinal absorption of orally taken medication [28]. Poorly bioavailable medications have TPSA > 140 A^{o} [29]. Another crucial parameter is the Lipinski rule of five., which states that orally bioavailable drugs should meet critical criteria such as molecular weight (M.Wt.) < 500Da, an octanol-water partition coefficient [Log P] less than 5, the number of

hydrogen bond donors less than 5, and number of hydrogen bond acceptors less than 10 [30].

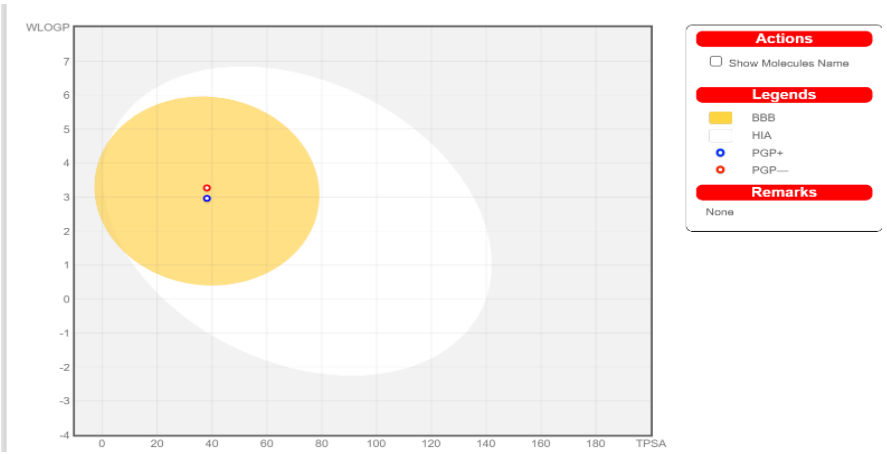


Figure 6. BOILED-Egg of compounds A-2 (red dot) and A-8 (blue dot). The molecule within yellow ovule's passively crosses the BBB. Molecules within white ovules can be absorbed through GIT. Blue dots are assigned to molecules that are considered P-glycoprotein substrates (PGP+), which can export from the central nervous system. The derivatives shown by red dots are those that P-glycoprotein is unable to remove from the CNS (PGP-).

Table 2 : Lipinski properties and permeation of fifteen Azetidin-2-one derivatives analyzed with Swiss-ADME.

COMP.	MW	H-B. A	H-B. D	TPSA	GI absorption	BBB permeant	P-gp Sub.	log Kp	Lipinski violations	Bio-availability
A-1	303.79	2	0	38.13	High	Yes	No	-6.64	0	0.55
A-2	379.88	2	0	38.13	High	Yes	No	-5.98	0	0.55
A-3	289.76	2	1	48.99	High	Yes	Yes	-6.52	0	0.55
A-4	324.21	2	0	23.55	High	No	Yes	-7.77	0	0.55
A- 5	400.3	2	0	38.13	High	Yes	No	-5.91	0	0.55
A- 6	310.18	2	1	48.99	High	Yes	Yes	-6.46	0	0.55
A-7	289.76	2	0	38.13	High	Yes	No	-6.81	0	0.55
A- 8	365.86	2	0	38.13	High	Yes	Yes	-6.15	0	0.55
A-9	275.73	2	1	48.99	High	Yes	No	-6.7	0	0.55
A- 10	334.76	4	0	83.95	High	No	No	-7.21	0	0.55
A-11	410.85	4	0	83.95	High	No	No	-6.54	0	0.55
A- 12	320.73	4	1	94.81	High	No	No	-7.09	0	0.55
A-13	319.79	3	0	47.36	High	Yes	No	-7.02	0	0.55
A-14	395.88	3	0	47.36	High	Yes	No	-6.35	0	0.55
A- 15	305.76	3	1	58.22	High	Yes	Yes	-6.9	0	0.55

Swiss-ADME tool for both compounds (A-2 and A-8), showing TPSA scores of 38.13 A. They exhibited a good bioavailability score, indicating their ability to reach blood circulation. The other proposed compounds possess TPSA below 94.81 and bioavailability for all ligands was 0.55 which means that all ligands can pass through systemic circulation, also they successfully met Lipinski rules as shown in table (2). GI absorption properties indicate the extent of drug that permeate through gastrointestinal tract into intestine after oral administration. In our study, since all suggested ligands have a high GI absorption score, good absorption is envisaged.

Protein-ligand complex system behavior is observed by molecular dynamic simulations via

RMSD, RMSF, and interactions of ligand with amino acid residues. Consequently, the highest-scoring ligand with the most convenient drug-likeness parameters was subjected to MD. The RMSD of compound A-2 was recorded for 50 ns, and the stability was assessed.

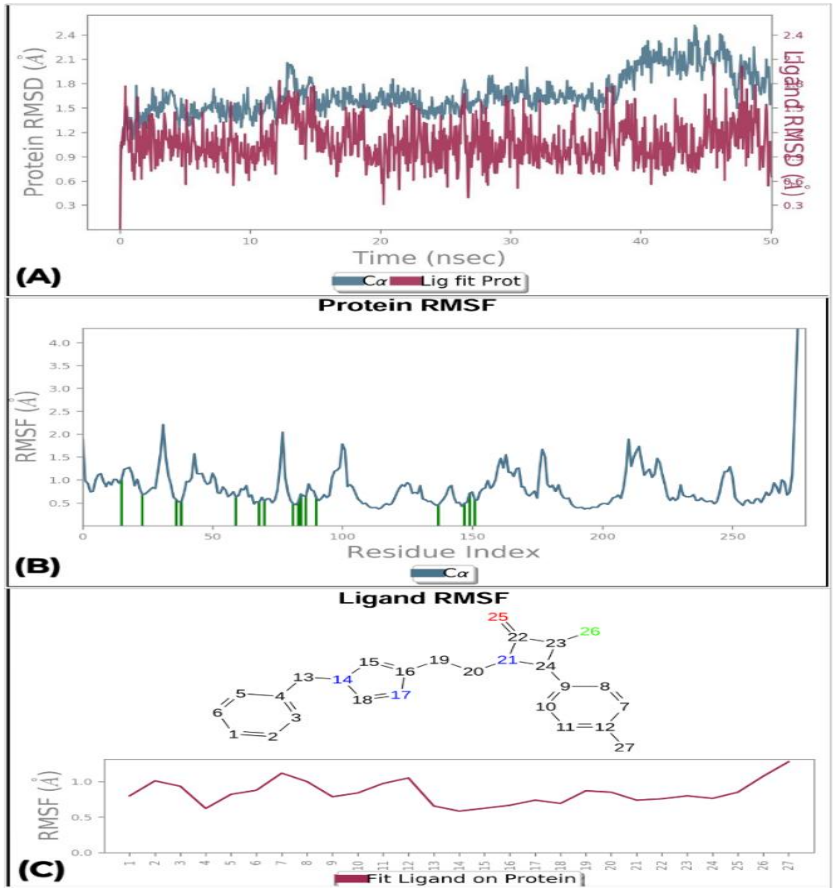


Figure 7. (A) RMSD plot of receptor-ligand complex in molecular dynamics study EGFR-compoundA-2 along 50 ns, (B) root mean square fluctuation RMSF of protein EGFR residue, (C) compound A-2 RMSF with EGFR protein.

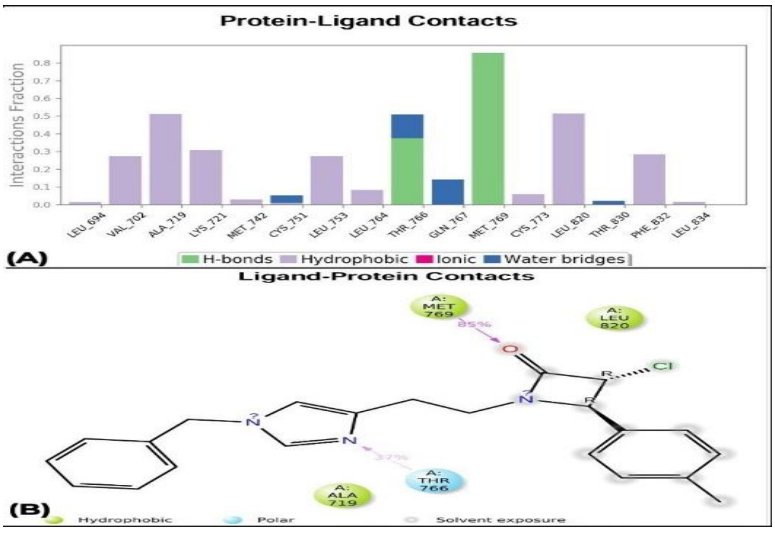


Figure 8. (A) histogram of compound A-2 contacts with EGFR protein throughout trajectory. (B) structure of compound A-2 with contacts inside active site of EGFR.

We found that the compound A-2-EGFR complex showed a deviation of approximately 1Å, which indicates a perfectly stable ligand that is aligned with protein and undergoes the same conformational variations along the course 50 ns of running. However, the deviation showed minimal fluctuation around 13 ns. Thus, compound A-2 was stabilized for the majority of the simulation time, the RMSD plot of receptor-ligand is depicted in Figure 7 (A).

The root mean square fluctuation (RMSF) of proteins gives an idea of the local variations along the protein chain. It determines the flexibility of the protein by identifying the rigid and flexible parts of protein. On the RMSF plot for residue, peaks represent regions of protein that exhibit the highest degree of fluctuation during the simulation. Usually, the tails (C- and N- terminal) tend to fluctuate more than other parts of the protein. While alpha helices and beta strands exhibit less fluctuation behavior than the loop regions, as the former is usually more rigid than the unstructured part of the protein. In the following Figure 7 (B), the green-colored vertical lines show the EGFR protein residues that interacted with compound A-2. The curve shows a significant number of interactions mostly in the lower fluctuations state, lower than 1Å, which makes it stable complex.

The ligand root mean square fluctuation (L-RMSF) details the changes in the ligand atom from their original position in the macromolecular structure. RMSF of ligand represented in Figure 7 (C), that determines the ligand's fluctuations broken down by atom, corresponding to the two-dimensional structure.

The RMSF of the ligand may provide insights on how ligand fragments interact with protein and their binding behavior. The protein-ligand complex is first aligned on protein backbone, and then L-RMSF is measured on the ligand heavy atoms. In the bottom panel of figure 7 (C), the line shows the ligand fluctuations. The majority of compound A-2 atoms demonstrate RMSF below 1 Å. while atom 27 show slightly higher fluctuations. Overall, RMSF shows acceptable values, which demonstrate how well compound A-2 fits with protein.

Furthermore, the interaction type between compound A-2 and EGFR is represented in figure 8 (A), which measures the binding affinity of the ligand. To take into consideration the dynamic fluctuations of the entire trajectory, stacked bar charts have been created. For example, hydrogen bonding between compound A-2 and MET769 residue and THR766 residue of EGFR protein was maintained at almost 90% and 38% during the simulation time. Whereas ALA719 and LEU 820 residues of protein maintained hydrophobic bonding with compound A-2 for 50% of the simulation time.

The MD result also showed the stability of interactions of compound A-2-EGFR complex, figure 8 (B). Respectively, the amino acid MET769 forms hydrophobic contact with oxygen of carbonyl group 85% of simulation time. Furthermore, the hydrogen bond forming amino acid with nitrogen atom of compound A-2 is THR766 with 37%. Ligand-protein contacts revealed that the binding of compound A-2 to the EGFR protein is stable.

4. Conclusions

Molecular docking is the most effective computational method for discovering new drugs with improved drug protein binding affinity and potency. The novelty of this study is the development of a series of azetidine-2-one derivatives and estimation of their potency profile. In fact, in comparison with erlotinib, our suggested compounds (A-2, A-8) demonstrate a better binding score within EGFR binding site, which indicates good antiproliferative activity. Furthermore, the physicochemical and pharmacokinetic properties of all suggested derivatives fulfill Lipinski's rule. The MDS study was conducted for compound A-2 and showed favorable values in RMSD, RMSF, and EGFR-ligand contacts. Finally, these newly designed compounds demonstrated encouraging results, making them a potential lead substance for the development of novel antiproliferative drugs; nevertheless, more pharmacological evaluation is necessary.

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