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In Silico Studies of a Novel Scaffold of Acetylsalicylic Acid Derivatives Against EGFR by Molecular Docking and Molecular Dynamics Simulations

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ABSTRACT

In this study, a molecular docking study was performed to propose the acetylsalicylic acid derivative 2-[(4-Acetylphenyl)carbamoyl]phenyl acetate (AMPBS) as a potential cancer candidate targeting the Epidermal Growth Factor Receptor (EGFR). The native ligand erlotinib was used as a control compound. The calculated docking score of -7.4 kcal/mol compared to the native ligand erlotinib of -7.0 kcal/mol of AMPBS compound revealed a promising anticancer activity. The stability of the complex was interpreted by careful analysis of the root mean square deviation (RMSD), root mean square fluctuations (RMSF) and mean hydrogen bond number (Hb) plots obtained from the MD trajectories. The ADMET properties of AMPBS were evaluated using relevant online tools. Drug-likeness studies showed that AMPBS is suitable for use in living organisms. It was predicted that AMPBS in the active pocket could be a promising inhibitor due to its high binding energy, interaction mechanism and retention in the active pocket.

Keywords: DFT, Molecular docking, Molecular dynamic, Acetylsalicylic acid, ADMET.

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Introduction

Cancer is a dangerous disease that threatens people's lives after cardiovascular disease and is very common worldwide [1]. There are many types of cancer, including melanoma, leukemia, colorectal, lung, ovarian, brain, prostate, kidney and breast cancer. Advances in technology, molecular biology and immunotherapy are enabling the development of treatment strategies for this relentless disease. Advances in clinical trials are playing a key role in the discovery of more effective treatments with fewer side effects, leading to an important transformation in cancer treatment.

EGFR is a receptor-type tyrosine kinase involved in cellular growth, division and survival [2, 3]. Activation of EGFR regulates cell growth and proliferation by affecting cellular signal transduction networks. 1M17 refers to the tyrosine kinase domain of EGFR [4]. This tyrosine kinase domain of EGFR is important for initiating intracellular signal transduction and regulating cell growth signals. This region plays a critical role for the activation of EGFR and affects various cellular processes within the cell. Because over-activation of EGFR has the potential to cause an uncontrolled increase in cell growth, drugs that target the tyrosine kinase activity of EGFR are used specifically in cancer therapy [5]. Therefore, EGFR tyrosine kinase inhibitors are targeted therapeutic agents specifically used in the treatment of certain types of cancer [6].

Medical research into cancer treatment has revealed that some known drugs may have unexpected benefits beyond their primary application. One of these drugs is acetylsalicylic acid (ASA), known as aspirin. ASA has long been recognized for its pain-relieving and anti-inflammatory properties [7]. However, recent studies have shown that the positive impact of ASA can extend to the field of cancer prevention and treatment. One of the main potential positive effects of ASA on cancer is its anti-inflammatory power [8, 9]. Inflammation has been recognized as the driving force behind the development and progression of cancer cells. ASA's powerful anti-inflammatory properties offer a unique opportunity to disrupt this process, potentially inhibiting the formation and spread of cancer cells [9, 10].

Moreover, studies show that ASA may go beyond simply inhibiting inflammation; it may also play an important role in breaking tumor cells' resistance to the body's immune system. This dual mechanism of action of ASA has led to an increased interest in the synthesis of ASA-based scaffolds with cancer-protective propertiesIn this context, Mohamed-Ezzat et al. synthesized AMPBS compound that can be used in the prevention of colorectal cancer and investigated *its in vitro* anti-proliferative activities. [11].

Computer-aided analysis methods have been widely used in recent years in the design of new drugs and elucidation of the interaction mechanisms and physicochemical properties of existing drugs with target receptors [12]. Computer-aided simulation studies and quantum mechanical calculation-based methods used for

this purpose contribute greatly to *in vivo* and *in vitro* studies in terms of cost and time savings.

In this study, we first evaluated the drug-likeness properties of the newly synthesized AMPBS compound. The next step was to elucidate the mechanisms of EGFR interaction with the kinase domain through molecular docking and molecular dynamics simulation (MDS) studies. Gefitinib, an EGFR tyrosine kinase inhibitor, was used as a control compound to interpret the results of molecular docking and molecular dynamics studies [13]. In summary, the study investigated the potential of the synthesized title compound as an anti-cancer compound against the target EGFR.

Materials and Methods

Density Functional Theory (DFT) Calculations

The basic level design of the molecular structure of AMPBS was carried out using Gaussview 5.0, a very useful tool for molecular modelling [14]. On the other hand, the molecular structure of the native ligand erlotinib (CID: 176870) was obtained from Pubchem database (https://pubchem.ncbi.nlm.nih.gov). The most stable structures corresponding to the ground states of both compounds and their properties such as HOMO-LUMO orbitals were theoretically calculated with Gaussian 09 software using quantum chemical calculation techniques [15] and given in Figures 1a and 1b. Quantum mechanical calculations were performed using DFT/B3LYP method and 6-31G basis set. HOMO-LUMO molecular orbital analysis was performed on the optimized structures to determine the reactivity of the compounds.

Molecular Docking Study

The three-dimensional crystal structure of the target protein for molecular docking, Tyrosine Kinase Domain from the EGFR (PDB: 1M17), was obtained from the Protein Data Bank database [16]. The drug-likeness properties of the compound were investigated with the help of SwissADME web tool (http://www.swissadme.ch). UCSF Chimera and AutoDock Vina tool, which are very useful in molecular docking studies to create complex structures of proteins and ligands and to visualise and analyse the interactions of molecular structures, were used together [17]. The drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the native ligand erlotinib and AMPBS were analyzed for prediction using SwissADME and pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm / prediction), respectively. For the docking study, components such as water molecules and hetatm were removed from the receptor by UCSF and polar hydrogen atoms and charges were assigned to the receptor. In this study, Discovery Studio 2019 Client molecular imaging software was used, which provides comprehensive tools to visualise molecular interactions, analyse binding affinities and assess the stability and quality of predicted ligand-receptor complexes.

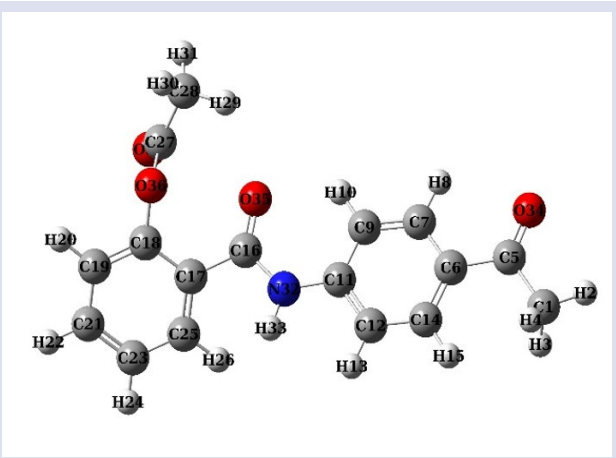
Molecular Dynamic Simulation Study

Unlike molecular docking studies, MDS studies allow protein-ligand interactions to be expanded biomolecularly, not at a single moment but over a certain time period [18]. In this study, MD Simulations were performed using Gromacs 2023.3 to analyse the interactions between protein and ligand. The input files and topology elements required for MD simulations were prepared using the CHARMM-GUI server [19] and the open access Swissparam web tool [20], respectively. MD simulations were performed in time steps of 2 fs for 50 ns. The RMSD, RMSF and Hb of protein-ligand interactions were analyzed by MDS.

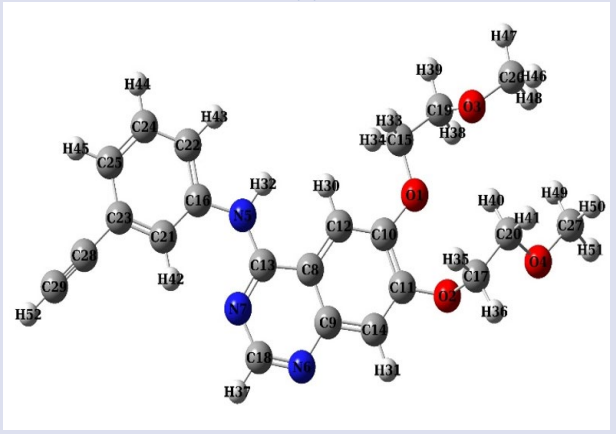
Results and Discussion

Density Functional Theory (DFT) Calculations

The first step in investigating the physicochemical, biological, and pharmacokinetic properties of compounds using quantum chemical calculations is the optimization of the compounds. The optimized structures of AMPBS and erlotinib compounds obtained using DFT/B3LYP method and 6-31G basis set are given in Fig. 1a and 1b, respectively.



(a)



(b)

Figure 1. Optimized molecular structures of AMPBS and erlotinib compounds by DFT/B3LYP method and 6-31G basis set, a) AMPBS, b) erlotinib

HOMO-LUMO molecular orbital analysis is a measure of intramolecular charge transfer through conjugated pathways between electrophilic and nucleophilic functional groups [21]. In molecules, the highest filled molecular orbit (HOMO) corresponds to nucleophilic centers, while the lowest empty molecular orbit (LUMO) corresponds to electrophilic centers. HOMO-LUMO molecular orbital energies are very important in the calculation of the reactivity of chemical systems as well as other global descriptors (Ionization potantial, electron affinity, chemical hardness/ softness, electronegativity, chemical potential, electrophilicity index). The HOMO-LUMO orbitals and related parameters for the compounds were calculated by DFT/B3LYP method and 6-31 G(d) basis set and are given in Table 1. In addition, the predicted 3D structure of the HOMO-LUMO orbitals is given in Fig 2. The energy gaps between the HOMO-LUMO orbitals characterize the biological reactivity of compounds. The HOMO energy levels of AMPBS and erlotinib compounds were -6.35 eV and -5.58 eV, respectively, while the LUMO orbital values were -1.72 and -1.31 eV. The energy gap between HOMO and LUMO was found to be 4.60 eV for AMPBS and 4.27 eV for erlotinib. Large energy gap indicates high stability and low chemical reactivity for molecules. The results showed that AMPBS had a higher kinetic stability than erlotinib.

Tablo 1. HOMO-LUMO orbital energies of the compounds and other calculated global descriptors.

Parameters (eV)	ligand	erlotinib	Parameters (eV)	ligand	erlotinib
LUMO energy	-1.72	-1.31	Chemical hardness (η)	2.30	2.14
HOMO energy	-6.32	-5.58	Chemical softness (S)	0.22	0.23
Energy Gap (ΔG)	4.60	4.27	Electronegativity (χ)	4.02	3.45
Ionization potantial (I)	6.32	5.58	Chemical potential (μ)	-4.02	-3.45
Electron Affinity (A)	1.72	1.31	Electrophilicity index (ω)	3.51	2.78

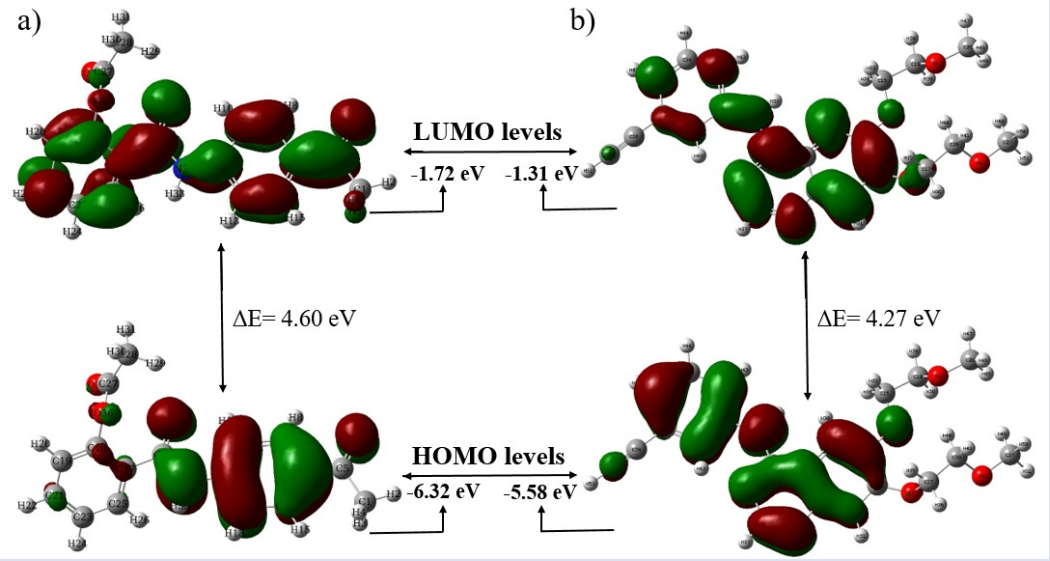


Figure 2. 3D drawing of HOMO, LUMO orbitals obtained from the optimized structure of compounds, a) AMPBS, b) erlotinib

Molecular Docking Study

The EGFR receptor coded 1M17 with a resolution of 2.60Å, which was retrieved from the protein databank for use in the molecular docking study, consists of several binding sites to which ligands can bind. TA pharmacophore model has been proposed for the binding pocket of EGFR, including hydrophobic region I, adenine site, hydrophobic region II, sugar pocket and phosphate binding site, respectively [22]. For AMPBS and Erlotinib compounds, the binding pocket of the EGFR receptor was identified using BIOVIA Discovery Studio software as the region containing the active residues Leu694, Ala719, Leu764, Thr766, Gln767, Leu768, Met769, Pro770, Phe771, Gly772, Leu820, Thr830 and Asp831. The region containing these active residues plays a key role in EGFR inhibition. Therefore, this region of the 1M17 receptor was used in the molecular docking study. As a result of the molecular docking study, the conformations with the best binding energy for protein-ligand interactions were selected and their h-bond, hydrophobic, electrostatic and Van der Waals interaction mechanisms were analyzed using BIOVIA Discovery Studio molecular visualization software. The interactions of the native ligand erlotinib and AMPBS compounds with the EGFR receptor are given in Fig. 3 and Fig. 4, respectively. Summary results of the interactions are presented in Table 2. As clearly seen in Table 2, the binding energies of AMPBS compound and erlotinib were found to be 7.4 kcal/mol and 7.0 kcal/mol,

respectively. The magnitude of the binding energy provides important information about the binding strength, efficacy and specificity of the drug to the target receptor. Lower (more negative) binding energy is one of the leading parameters of a more effective and specific drug development process [23]. Fig. 3 and Fig. 4 showed that both ligands travelled to the active site of the receptor and placed there. Erlotinib showed conventional hydrogen bond with amino acids Lys721 and Met769 from the active site residues of 1M17 and C-H bond interactions with residues Arg817 and Gln767 according to the interaction mechanism given in Fig. 3. Pi-Pi-stacked with Phe699, Pi-Alkyl hydrophobic interactions with residues Phe699, Val702, Lys721 and Leu820 and It showed 2 Pi-Anion electrostatic interactions with Asp831 residue. On the other hand, Van der Waals interactions were observed between Thr830, Ala719, Leu768, Leu694 and Cys773.

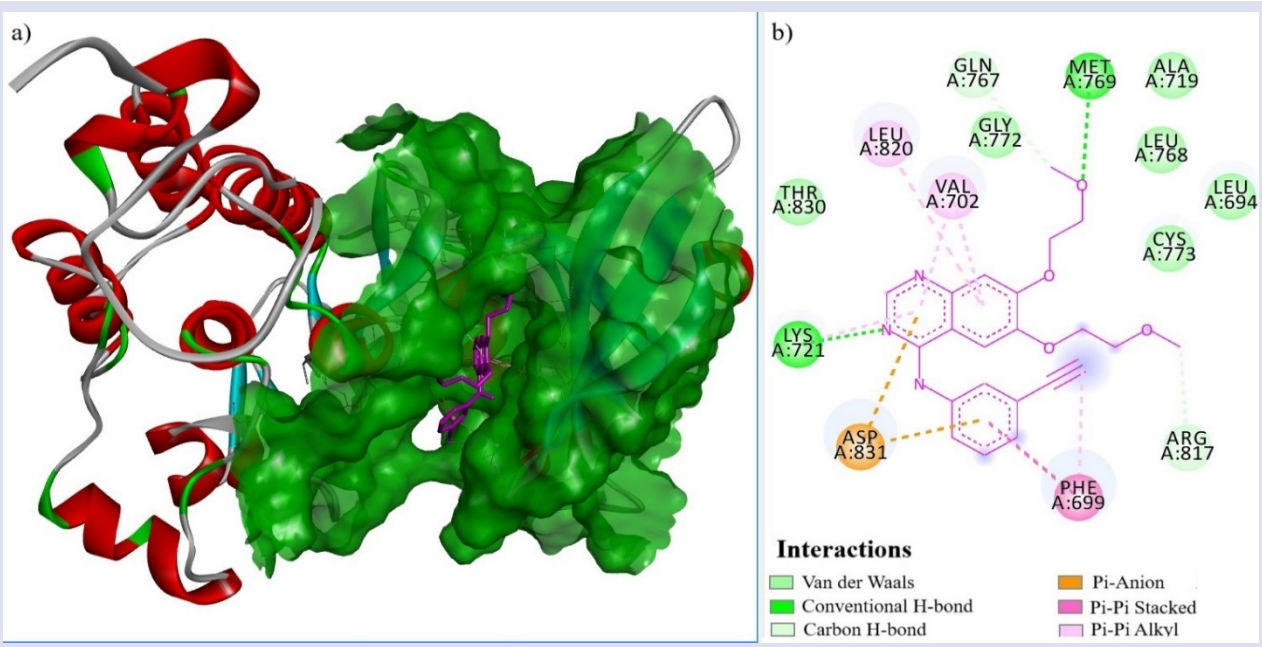


Figure 3. Post docking interactions between active site residues of EGFR with erlotinib, a) 3D solid surface drawings of the interaction, (b) 2D interaction diagram of ligand with EGFR protein.

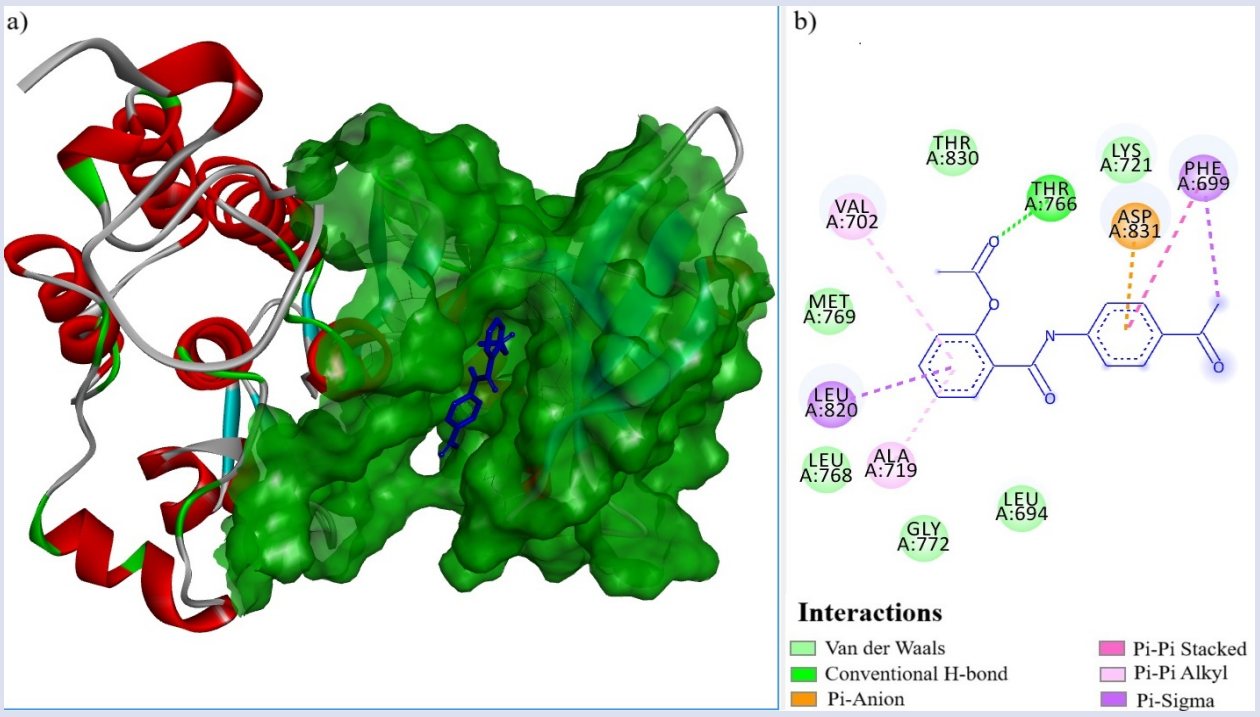


Figure 4. Post docking interactions between active site residues of EGFR with AMPBS, a) 3D solid surface drawings of the interaction, (b) 2D interaction diagram of ligand with EGFR protein.

AMPBS in Fig. 4 showed conventional hydrogen bond with residue Thr766 in the active site of 1M17, Pi-Pi-stacked with residue Phe699, Pi-Sigma with residues Leu820, Pi-Sigma with residues Phe699, Pi-Alkyl hydrophobic interaction with residues Val702 and Ala719 and 2 Pi-Anion electrostatic interactions with residue Asp831. On the other hand, Van der Waals interactions were observed between Thr830, Leu694, Lys721, Gly772, Leu768 and Met769.

Table 2. Summarative results of Post docking interactions between EGFR with ligands

Protein	Ligands	ΔG (kcal/mol)	Hydrogen Bond interactions (Å)	Hydrophobic interaction (Å)	Electrostatic interaction (Å)	Van der Waals
1M17	Erlotinib	-7.0	Conventional H-Bond Lys721(3.04), *Met769(2.92) Carbon H-Bond Arg817(3.70), *Gln767(3.72)	Pi-Pi stacked Phe699(3.80) Pi-Alkyl Phe699(5.18), Val702(4.80-4.13), Lys721(5.05), *Leu820(5.37)	Pi-Anion *Asp831(3.64-3.63-3.96)	Ala719, Leu768, Leu694, Cys773, Thr830
	AMPBS	-7.4	Conventional H-Bond *Thr766(2.75)	Pi-Pi stacked Phe699(4.83) Pi-Sigma *Leu820(3.75), Phe699(3.90), Pi-Alkyl Val702(5.40), *Ala719(4.75)	Pi-Anion *Asp831(3.58-4.50)	Thr830, Lys721, Leu694, Gly772, Leu768, Met769

*Active residues of 1M17

Drug-Likeness and ADMET Properties

Drug synthesis studies to be used for the treatment of many diseases that threaten living health are both time consuming and very costly. In this respect, the combination of computer technologies and multi-disciplinary approaches has made significant contributions to new drug designs. Although drug-likeness studies do not provide conclusive results, they provide useful predictions for the early stages of new drug design. There are some criteria used in drug-likeness studies and developed based on previous drug designs. One of the most common of these is the Lipinsky rule of five [24]. The drug-likeness of erlotinib and AMPBS compounds were evaluated within the framework of Lipinski's five criteria and presented in Table 3. It is clear from Table 3 that there are no violations of the Lipinski criterion for either compound that would preclude their use in living organisms.

Table 3. Drug-likeness analysis of AMPBS and Erlotinib.

Lipinski's five criteria	Accepted range	Erlotinib		AMPBS	
		Value	result	Value	result
Molecular Weight (Da) (MW)	≤500	393.44	✓	293.3	✓
Num. H-bond donors	≤5	1	✓	1	✓
Num. H-bond acceptors	≤10	6	✓	4	✓
LogP	≤5	3.67	✓	2.43	✓

According to these results, AMPBS is expected to be permeable through the cell membrane, easy absorption, transport and distribution [25]. ADMET study was performed to evaluate these properties. ADMET predictions performed through the web-based online pkCSM tool for erlotinib and AMPBS compounds are summarized in Table 4. Absorption part of the ADMET study both compounds exhibited excellent intestinal (human) absorbance values around 95%. In the distribution part of the ADMET study, the blood brain barrier (BBS) penetrability values of erlotinib and AMPBS compounds were found to be -0.465 and -0.16, respectively, and the central nervous system (CNS) penetrability values were found to be -3.418 and -2.212,

respectively. Accepted penetrability values for BBS and CNS are expected to be between -1 and 0.3 and -3 and -2, respectively [26]. Toxicity analysis is an important and guiding method for safer new drug designs. When the Ames toxicity values of the compounds in the table are analyzed, it is estimated that erlotinib is not toxic but AMPBS may be toxic. On the other hand, the opposite is the case in hepatotoxicity values. Cytochrome P450 (CYP) enzymes form the major metabolizing enzyme system in humans and are responsible for the chemical changes that many molecules synthesized as drugs undergo in the body. Cytochrome P450 (CYP) enzymes (CYP2D6, CYP2C9, CYP2C19, CYP3A4, CYP1A2) constitute the major metabolising enzyme system in humans and are responsible for the chemical changes that many molecules synthesized as drugs undergo in the body. Of these enzymes, CYP3A4 is the most common and important in terms of interactions [27]. Metabolism results showed that both compounds were substrates of CYP3A4, while only erlotinib was an inhibitor of CYP3A4. The term clearance refers to the rate of excretion of the drug from the body (Clearance n High: >15 mL/min/kg; moderate: 5-15 mL/min/kg; low: <5 mL/min/kg [28] A high clearance indicates that the drug is cleared from the body rapidly, while a low clearance means that the drug is cleared more slowly. This is important in determining how long a drug can be effective and in adjusting its dosage. The clearance values of the compounds in Table 4 showed that the compounds have a high persistence in the body.

Table 4. ADMET analysis results obtained by using pkCSM tools.

Properties	Compounds		Properties	Compounds	
Absorption	Erlotinib	AMPBS	**Distribution**	Erlotinib	AMPBS
Water solubility (log mol/L)	-5.081	-3.693	VDss (human) (log L/kg)	-0.02	-0.036
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.185	1.183	Fraction unbound (human) (Fu)	0.05	0.102
Human intestinal absorption (HIA+, %)	95.43	94.432	BBB permeability (log BB)	-0.465	-0.16
Skin Permeability (log Kp)	-2.758	-2.877	CNS permeability (log PS)	-3.418	-2.212
P-glycoprotein substrate	No	No	**Metabolism**		
P-glycoprotein I inhibitor	Yes	No	CYP2D6 substrate	No	No
P-glycoprotein II inhibitor	Yes	No	CYP3A4 substrate	Yes	Yes
Toxicity			CYP1A2 inhibitior	No	Yes
AMES toxicity	No	Yes	CYP2C19 inhibitior	Yes	Yes
Max. tolerated dose (human) (log mg/kg/day)	0.714	0.411	CYP2C9 inhibitior	Yes	No
hERG I inhibitor	No	No	CYP2D6 inhibitior	No	No
hERG II inhibitor	Yes	No	CYP3A4 inhibitior	Yes	No
Oral Rat Acute Toxicity (LD50) (mol/kg)	2.499	1.988	**Excretion**		
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.183	1.555	Total Clearance (log ml/min/kg)	0.63	0.314
Hepatotoxicity	Yes	No	Renal OCT2 substrate	No	No
Skin Sensitization	No	No			
T. Pyriformis toxicity (log ug/L)	0.34	1.331			
Minnow toxicity (log mM)	-1.76	1.088			

Molecular Dynamic Simulation

Important information on the conformation of ligand molecules in the active cavity of the target receptor and the interactions that occur were obtained from molecular docking studies. MDS studies were performed using the Gromacs 2023.3 package at time steps of 2 fs for 50 ns to understand the behavior of erlotinib and AMPBS compounds in the active site and the stability of receptor-ligand interactions. In this study, RMSD, RMSF and Hb plots were generated and the results analyzed to investigate the actual mechanisms of the interactions.

RMSD analysis is used to obtain information about the conformational stability of pharmacological molecules [29]. The RMSD plot in Fig. 5a clearly shows that erlotinib exhibited a stable behavior around the equilibrium point around 0.67 nm after 20 ns until the end of the simulation, initially showing high fluctuations that gradually decreased. On the other hand, the RMSD plot in Figure 3b shows that AMPBS behaved unstably around 0.8 nm after 20 ns until 40 ns and exhibited a stable behavior after 40 ns until the end of the simulation. The RMSD plots obtained from MDS clearly showed that erlotinib

oscillated stably around the equilibrium point, reaching a plateau in the first 10 ns, unlike AMPBS. On the other hand, RMSD graphs revealed that both compounds exhibited similarly stable movements around the equilibrium point at the end of the MDS. For the analysis of the positional fluctuations of protein residues, the RMSF plot obtained from MDS is very useful. In this study, the RMSF plot was plotted against each atom based on the MDS trajectory for each of the atom mobility complexes and is given in Fig. 5b. Figure 5b clearly shows that the fluctuation profiles of the complexes are quite overlapping. This implies that AMPBS can form a stable complex with EGFR and inhibit it like the native ligand erlotinib. It is well known that hydrogen bonding interactions play an important role in the structural stability of proteins. It provides information about the number of hydrogen bonds formed in the complex. As seen in Fig. 5c, the maximum number of hydrogen bonds formed in the erlotinib and AMPBS complexes are 3 and 2, respectively .

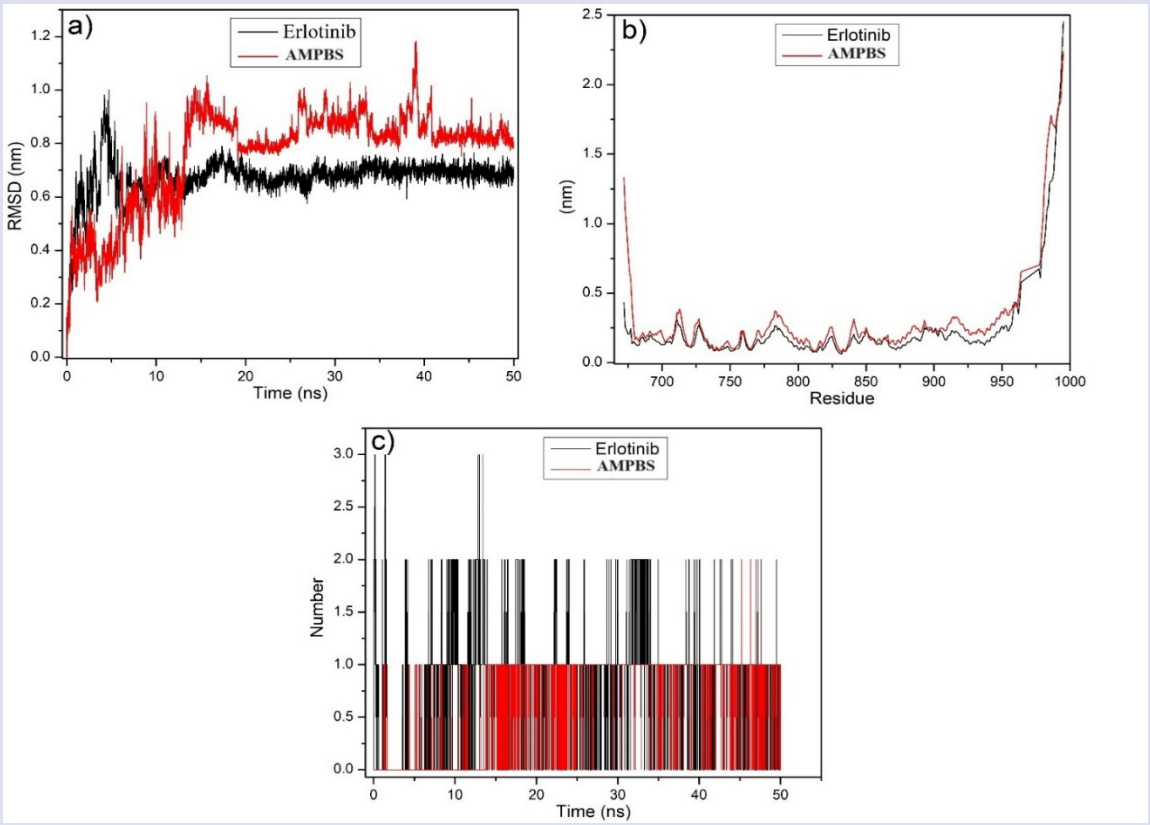


Figure 5. a) RMSD of protein and ligands; b) RMSF of the amino acid residues; c) H-bond count of the protein-ligands complex.

Conclusion

In this study, molecular docking, ADMET and MDS studies were used to identify possible therapeutic inhibitors of pharmacological importance that are predicted to show antiproliferative activity against EGFR. The native ligand erlotinib was used as a control compound. Chemical stability of the compounds was calculated by DFT calculations. The results obtained from ADMET analysis strongly indicated that the studied compounds can behave like a drug and exhibit remarkable biological activities. In addition to ADMET studies, when drug-likeness was evaluated according to Lipinski criteria, the compounds were found to obey Lipinski's rule without any violation. In the docking study, both molecules travelled to the active site and docked there. In addition, when the binding scores obtained were compared, it was observed that AMPBS had higher binding energy than erlotinib. The results of the docking studies were evaluated by MDS, especially the RMSF analysis almost overlapped with the control compound. In conclusion, the positive results obtained from *in silico* studies for AMPBS often encourage *in vitro* (laboratory) and *in vivo* (animal) testing for a new drug compound. These studies are necessary to confirm the biological activity, safety and pharmacokinetic properties of the compound. Successful results can lead to the initiation of clinical trials and ultimately the development of the compound as a medicine.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgment

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