Molecular Docking Studies of Benzoxazole derivatives through ADMET properties as potential agents for lung cancer

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Abstract

Cancer refers to a group of disorders marked by the rapid and uncontrolled proliferation of abnormal cells, which can invade surrounding tissues and metastasize to distant parts of the body. Among various molecular targets, mutations in the epidermal growth factor receptor (EGFR) have been increasingly recognized as pivotal in the pathogenesis and progression of non-small cell lung cancer (NSCLC). Although EGFR tyrosine kinase inhibitors (EGFR-TKIs) have been developed as targeted therapies, the emergence of drug resistance remains a significant clinical challenge. In light of these limitations, benzoxazole derivatives (BPR1–BPR5) were evaluated using computational approaches to assess their drug-likeness and binding affinities. The evaluation was based on key pharmacokinetic parameters, including molecular weight (<500 Da), LogP (<5), hydrogen bond donors (<5), hydrogen bond acceptors (<10), and the number of rotatable bonds (<10). All compounds satisfied these criteria, indicating favourable drug-like properties. Molecular docking studies further demonstrated strong binding affinities of the derivatives to the target protein. Notably, compound BPR1 exhibited a binding energy of –8.8 kcal/mol, surpassing that of the reference drug gefitinib (–8.1 kcal/mol). In addition to its superior binding affinity, BPR1 also displayed promising pharmacokinetic and toxicity profiles, highlighting its potential as a lead compound for further development.

KEYWORDS: *ADMET, cancer, in silico, molecular docking, NSCLC*

INTRODUCTION

Cancer is a group of disorder attributed by the rapid and uncontrolled proliferation of abnormal cells, which have the potential to invade nearby tissues and metastasize to distant parts of the body[1]. In the 21st century, cancer has emerged as the most prevalent noncommunicable disease (NCD), contributing significantly to global mortality and acting as a major barrier to increased life expectancy worldwide[2].

The American Cancer Society has projected in 2024, approximately 2,001,140 new cancer cases and 611,720 cancer-related deaths will occur in the United States. This corresponds to an average of about 5,480 new diagnoses and 1,680 deaths each day. Among men, prostate cancer, lung cancer, bronchus cancer and colorectal cancer account for almost half (48%) of all incident cases where prostate cancer alone accounts for 29% of diagnoses. In women, breast cancer, lung cancer, and colorectal cancer account for 51% of all new diagnoses. Breast cancer alone accounts for 32% of cases[3]. Lung and bronchus cancer is projected to be one of the most common cancers diagnosed in 2024, with approximately 340 people dying each day from lung cancer nearly 2.5 times more than those who die from colorectal cancer, which ranks second in cancer deaths. An estimated 101,300 out of 125,070 lung cancer deaths (81%) will be directly linked to active tobacco consumption, with passive exposure contributing to an additional 3,500 fatalities. Non-small-cell lung cancer (NSCLC) accounts for roughly 85% of all lung cancer cases and continues to be a leading cause of cancer-related deaths globally. Currently, the most effective treatment option is surgical resection, which is often not feasible

in advanced stages. Chemotherapy, especially platinum-based drugs, remains a cornerstone of treatment but is associated with significant toxicity due to damage to both healthy and cancerous cells [4].

A major advancement in the understanding of non-small cell lung cancer (NSCLC) was achieved in 2004 with the discovery that mutations in the epidermal growth factor receptor (EGFR) function as key oncogenic initiators**.** This discovery profoundly advanced the molecular characterization of lung cancer and laid the foundation for the development of targeted therapies directed at EGFR mutations. EGFR tyrosine kinase inhibitors (EGFR-TKIs), like gefitinib and erlotinib, have subsequently shown considerable clinical effectiveness in patients with EGFR-mutant NSCLC. However, the development of resistance to these therapies remains a major challenge. Resistance mechanisms include secondary EGFR mutations (e.g., T790M), activation of alternative signaling pathways (e.g., c-Met, HGF, AXL), alterations in downstream signaling (e.g., K-RAS mutations, PTEN loss), impaired apoptotic pathways (e.g., BIM deletion polymorphism), histological transformation, and enhanced drug efflux through ATP-binding cassette (ABC) transporters[5].

Heterocyclic compounds are cyclic organic structures containing at least one hetero atoms as play a vital role in various branches of chemistry, including pharmaceuticals, materials science, photochemistry, agriculture and dyes[6]. Heterocycles are crucial scaffolds in numerous marketed anticancer agents. Remarkably, almost two-thirds of the novel anticancer drugs approved by the FDA between 2010 and 2015 feature heterocyclic rings in their structures. Remarkably, almost two-thirds of the new anticancer medications authorized by the FDA between 2010 and 2015 contain heterocyclic rings within their molecular structures[7].

Among these, benzoxazole stands out as a key aromatic heterocyclic compound composed of a fused benzene and oxazole ring, with the molecular formula C_7H_5NO. It is isosteric with other biologically relevant heterocycles like indole and benzothiazole. Its structural similarity to nucleic acid bases such as guanine and adenine enables it to interact with biopolymers in biological systems, contributing to a diverse array of pharmacological activities. The presence of nitrogen and oxygen atoms in the benzoxazole ring influences its electron distribution, enhancing its stability and making it an attractive candidate in materials science, particularly in the fields of organic semiconductors and fluorescent probes[7-9]. Benzoxazole derivatives exhibit a wide spectrum of biological activities, including antimicrobial[10], antibacterial[11], antifungal[12], anticancer[4], anti-inflammatory[13], and antiviral[14] properties. In modern medicinal chemistry, benzoxazole is important scaffolds for designing ligands with enhanced affinity and selectivity for specific biological targets. These beneficial interactions include π–π stacking, cation–π interactions, hydrogen bonding, and hydrophobic effects. Due to these properties, benzoxazole derivatives are commonly incorporated into ligands that target various receptors and enzymes involved in disease pathophysiology, including cancer. Their distinctive features position them as promising candidates for targeted cancer therapies[4,8].

This highlights the importance of new, more effective, and targeted therapeutic drugs. Benzoxazole derivatives have shown interesting biological activity and structural properties,

which may make them a target for cancer treatment. In this study, new benzoxazole derivatives are screened with *in-silico* methods like molecular docking and ADMET prediction to identify potential lead compounds for the therapy of non-small cell lung cancer.

MATERIALS AND METHOD

A. Drug likeliness prediction

Early evaluation of pharmacokinetic profile has been demonstrated to substantially reduce the incidence of pharmacokinetics-related failures in subsequent clinical trials[15]. In the present study, the drug likeliness properties of the compounds were accessed using the Swiss ADME prediction tool, which offers five distinct rule-based filters encompassing a broad spectrum of physicochemical parameters indicative of drug-likeness[16]. Furthermore, the potential toxicological profiles of the compounds were evaluated using the ProTox 3.0 software[17].

B. Selection of protein: -

The three-dimensional structure of the protein (PDB ID: 4WKQ), with a resolution of 1.85 Å, was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) based on literature reports and saved in PDB format[18].

FIG.1. PDB ID: 4WKQ

C. Preparation of protein: -

The protein was prepared for docking using Auto dock tools 1.5.7 by removing water molecules and the native ligand, adding polar hydrogens and Gasteiger charges, and finally saving the processed protein in PDBQT format[18].

D. Preparation of ligand: -

The 2D structures of benzoxazole derivatives (BPR1–BPR5) were sketched in ChemDraw and are presented in **table 1.** These were then converted into 3D models and optimized through energy minimization. The final structures were saved on PDBQT format for molecular docking studies[19]. Gefitinib is a standard drug used for treatment of oesophageal cancer which is obtained from PubChem.

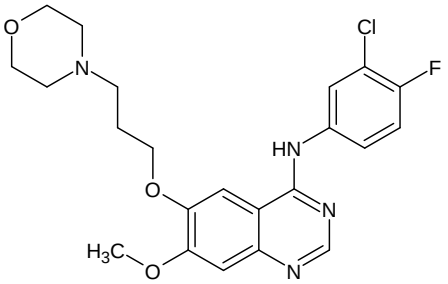
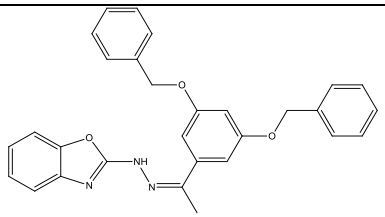
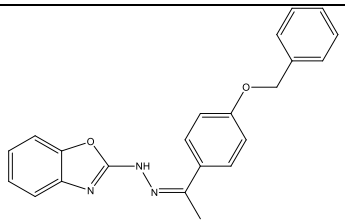
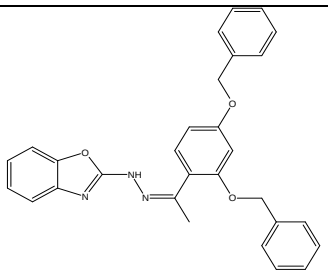
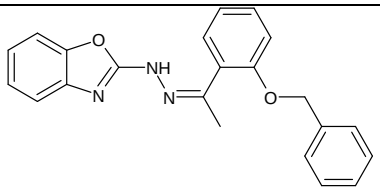
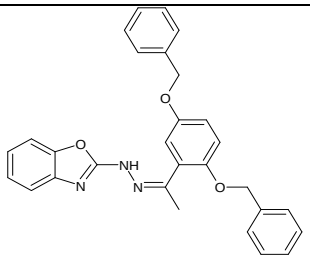


FIG.2. MOLECULAR STRUCTURE OF GEFITINIB

TABLE 1: STRUCTURES OF SKETCHED LIGANDS

Compound's code	Structure
BPR1	
BPR2	
BPR3	
BPR4	
BPR5	

E. Molecular docking: -

Molecular docking refers to a computational technique employed to predict interactions between ligands and macromolecule, thereby supporting virtual screening, compound prioritization, and lead optimization in the drug discovery process. In the present study, molecular docking study was performed using Auto Dock Tools version 1.5.7, following a systematic protocol. Initially, the ligand structures were optimized and saved in PDBQT format. Subsequently, the target protein was prepared and also saved in PDBQT format. A grid box was generated to define the active site for docking simulations. Docking of the ligand and protein was conducted using AutoDock, and the resulting interactions were analyzed and visualized in both 2D and 3D using BIOVIA Discovery Studio Visualizer[20].

RESULTS AND DISCUSSION

A. Evaluation of drug likeliness properties

The pharmacokinetic properties and drug-like characteristics of the novel compounds BPR1–BPR5 were accessed using the SwissADME tool http://www.swissadme.ch/ , with the results summarized in **table 2**. Compounds exhibiting a Topological Polar Surface Area (TPSA) of less than 140 Å and fewer than 10 rotatable bonds (n-ROTB) are generally considered to possess favourable molecular flexibility, which enhances their interaction with biological targets. As shown in Table 2, all five compounds exhibit TPSA values below 140 Å and n-ROTB values under 10, indicating a favourable degree of conformational flexibility for receptor binding. Additionally, the predicted miLogP values for all compounds are below 5, suggesting good potential for membrane permeability. Each compound also possesses an acceptable count of hydrogen bond donors (HBDs) and acceptors (HBAs), remaining below the standard thresholds of 5 and 10, respectively. Moreover, the molecular weights of all compounds are under 500 Da. These characteristics collectively indicate compliance with Lipinski's Rule of Five, further supporting their potential as orally bioavailable drug candidates. Collectively, these physicochemical properties satisfy Lipinski's Rule of Five**,** indicating their potential as orally bioavailable bioactive molecule.

TABLE 2: ADME PREDICTION OF THE COMPOUNDS

Compound code	***Molar refractivity***	***Water solubility***	***GI absorption***	***BBB permeant***	**P-glycoprotein substrate**	**CYP 1A2 inhibitor**	**CYP 2C19 inhibitor**	**CYP 2C9 inhibitor**	**CYP 2D6 inhibitor**	**CYP 3A4 inhibitor**	**Log *K*$_p$ (skin permeation)**
BPR1	138.25	Poorly	High	No	No	No	Yes	Yes	Yes	Yes	-4.25cm/s

		soluble									
BPR2	107.27	Poorly soluble	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-4.65cm/s
BPR3	107.27	Poorly soluble	High	No	No	No	Yes	Yes	No	Yes	-4.25cm/s
BPR4	112.28	Poorly soluble	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-4.65cm/s
BPR5	107.27	Poorly soluble	High	No	No	No	Yes	Yes	Yes	Yes	-4.25cm/s

TABLE 3: *IN-SILICO* PHYSICOCHEMICAL PROPERTIES

Compound Code	***MW (g/mol)***	***miLogP***	***Num. Arom. Heavy atoms***	***Num. H-bond acceptors***	***Num. of H-bond donors***	***Num. Rotatable bonds***	***TPSA***	***n violation***
BPR1	463.54	4.34	27	6	1	9	68.89	0
BPR2	357.41	3.81	21	5	1	6	59.66	0
BPR3	463.54	4.95	27	6	1	9	68.89	0
BPR4	357.41	3.41	21	5	1	6	59.66	0
BPR5	463.54	4.38	27	6	1	9	68.89	0

B. Toxicity prediction

Toxicity evaluation of the compounds was conducted using the ProTox 3.0 https://tox.charite.de/protox3/index.php?site=home online prediction tool. The estimated oral LD_{50} values (rat model) were 1190 mg/kg**,** placing the compounds in toxicity Class IV according to the Globally Harmonized System (GHS), which indicates that they are potentially harmful if swallowed. The predictions indicated that the compounds are inactive for carcinogenicity, mutagenicity and cytotoxicity whereas, carcinogenicity and hepatotoxicity were predicted to be active**.** These outcomes highlight the need for further toxicological evaluation to confirm and better understand the potential adverse effects associated with these compounds.

TABLE 4: *IN-SILICO* TOXICITY STUDIES OBTAINED FROM PRO-TOX 3.0

Compound code	*Predicted LD50 (mg/kg*	*Predicted Toxicity Class*	*Hepatotoxicity*	*Carcinogenicity*	*Immunotoxicity*	*Mutagenicity*	*Cytotoxicity*
BPR-1	670	4	Active	Active	Inactive	active	Inactive
BPR-2	670	4	Active	active	inactive	active	Inactive
BPR-3	670	4	Active	active	inactive	active	Inactive
BPR-4	670	4	Active	active	inactive	active	Inactive
BPR-5	670	4	Active	active	inactive	active	Inactive

***TABLE 5:* BINDING ENERGY**

Compound code	**Binding affinity (kcal/mol)**	**Conventional hydrogen bond interactions**	**Bond length**	**Hydrophobic interactions**
BPR1	-8.8	LYS -745 ASP -855	2.39 2.78	ASP -880, ARG -841, VAL -726
BPR2	-8.2	LYS -745 ASP -855	2.21 2.86	LEU -718, LEU -844, ALA -743, VAL -726
BPR3	-8.5	LYS -745	2.85	ARG -841, ASP -800, VAL -726, LEU -718, ALA -743
BPR4	-8.4	LYS -745	3.03	MET -766, VAL -726, ALA -743, LEU -718
BPR5	-8.7	LYS -745 ASP -855	2.75 2.02	MET -766, VAL -726, ALA -743, LEU -718, LEU -844, LEU -799, ARG -841
Standard drug (Gefitinib)	-8.1	LYS -745 ASP -855 ASP -800	2.46 2.17 2.08	LEU -844, VAL -726, GLY -719

C. MOLECULAR DOCKING STUDY:

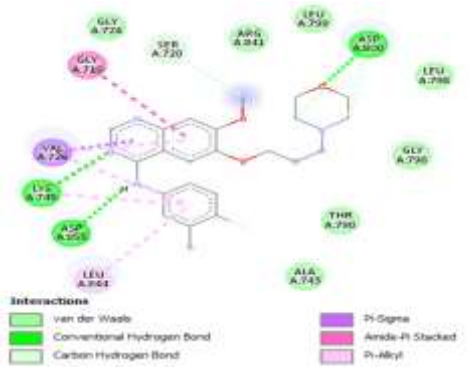
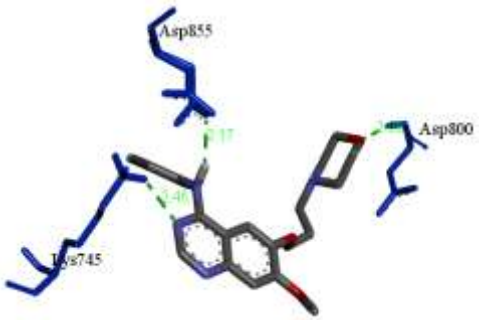
A molecular docking study was conducted using the target protein obtained from the RCSB Pdb (PDB ID: 4WKQ), which possesses a resolution of 1.85 Å. The docking analysis of benzoxazole derivatives was performed using gefitinib as the reference compound for this specific target. The binding energies of the tested derivatives are presented in table 2, and the corresponding 2D and 3D interaction profiles are depicted in figure 1. All the evaluated derivatives demonstrated higher binding affinities than the reference drug, gefitinib, which showed a binding energy of -8.1 kcal/mol. Gefitinib formed hydrogen bonds with residues LYS

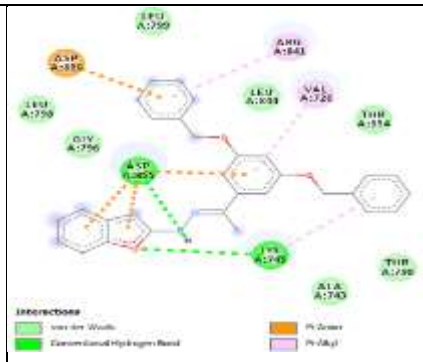
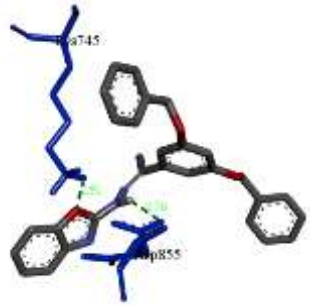
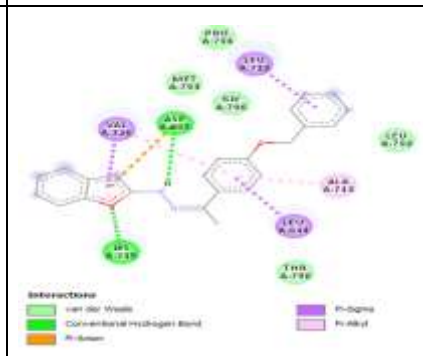
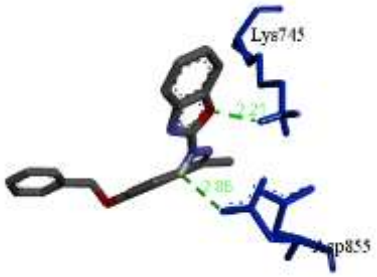
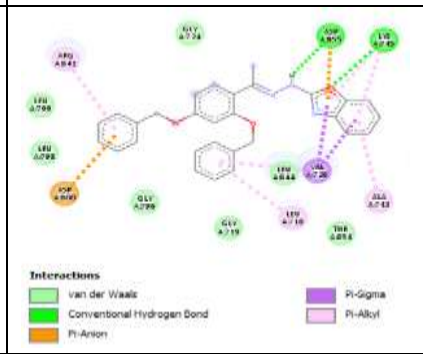
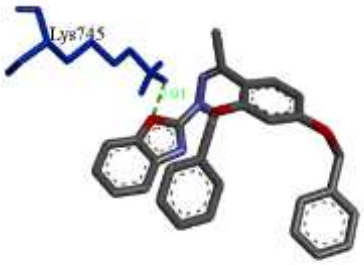
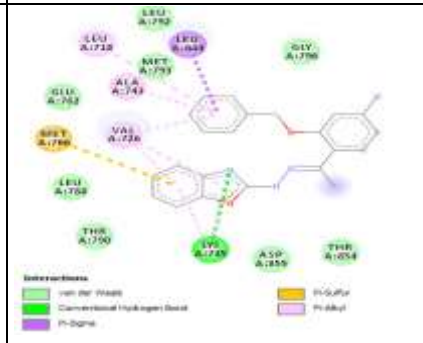
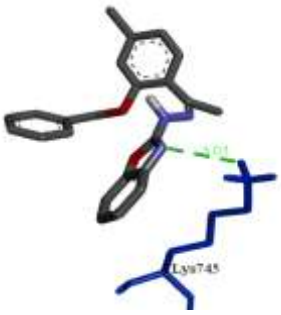
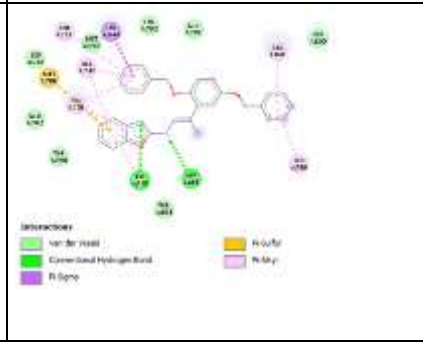
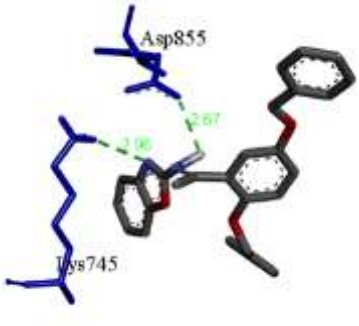
-745, ASP -855, and ASP -800, with bond lengths of 2.46 Å, 2.17 Å, and 2.08 Å, respectively, along with hydrophobic interactions involving LEU -844, VAL -726, and GLY -719.

Among the derivatives, compound BPR1 displayed the strongest binding energy with a docking score of -8.8 kcal/mol. It formed hydrogen bonds with LYS -745 and ASP -855 at bond lengths of 2.39 Å and 2.78 Å, respectively, and exhibited hydrophobic interactions with ASP -880, ARG -841, and VAL -726. BPR2 showed a binding score of -8.2 kcal/mol, forming hydrogen bonds with LYS -745 and ASP -855 (bond lengths: 2.21 Å and 2.86 Å), and hydrophobic interactions with LEU -718, LEU -844, ALA -743, and VAL -726. Compound BPR3 demonstrated a binding energy of –8.5 kcal/mol, forming a hydrogen bond with LYS -745 (2.85 Å) and hydrophobic interactions with ARG -841, ASP -800, VAL -726, LEU -718, and ALA -743. BPR4 exhibited a binding energy of –8.4 kcal/mol with a hydrogen bond to LYS -745 (3.03 Å) and hydrophobic interactions with MET A:766, VAL -726, ALA -743, and LEU -718. Lastly, BPR5 achieved a binding energy of –8.7 kcal/mol, forming hydrogen bonds with LYS -745 and ASP -855 (bond lengths: 2.75 Å and 2.02 Å), and hydrophobic interactions with MET -766, VAL -726, ALA -743, LEU -718, LEU -844, LEU -799, and ARG -841.

With the exception of compound BPR2, which demonstrated a binding score comparable to that of gefitinib, all derivatives showed improved docking scores. Notably, all the compounds formed conventional hydrogen bonds with LYS -745 and ASP -855 key interactions also observed with gefitinib. This suggests that the synthesized derivatives may adopt a similar binding mode to the target receptor, implying a potential similarity in biological activity.

TABLE 6: DOCKING INTERACTIONS

Compound code	2D Interaction	3D Interaction
Standard drug (Gefitinib)		

BPR1		
BPR2		
BPR3		
BPR4		
BPR5		

CONCLUSION

In this study, a series of benzoxazole derivatives were systematically investigated using various *in silico* methods, including molecular docking, toxicity prediction, and physicochemical property evaluation. The docking analysis revealed that all compounds exhibited binding affinities comparable to the standard drug against the selected biological target (PDB ID: 4WKQ). Among them, compound BPR1 showed the strongest binding affinity, with a binding energy of –8.8 kcal/mol, which is higher than that of the reference drug gefitinib (–8.1 kcal/mol), indicating significant potential for bioactivity and the benzoxazole derivatives form conventional hydrogen bonds with the same amino acid residues, LYS -745 and ASP -855, confirming the potential use of these derivatives for lung cancer treatment. These findings indicate the potential of these compounds as candidates for lung cancer treatment, specifically non-small cell lung cancer (NSCLC). Nevertheless, further structural modifications are recommended to enhance the toxicity profile before progressing to experimental validation through in vitro and in vivo studies.

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