In Silico Screening of Potential Inhibitors of the Epidermal Growth Factor Receptor Kinase using Benzimidazole, Benzoxazole, Imidazole, and Tetrazole Derivatives

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Background: Small molecule compounds are docked into receptor binding sites and the binding affinity of the complex is calculated using the structure-based drug design technique. Precise and quick docking processes, as well as the capacity to examine binding geometries and interactions, are required for a full knowledge of the structural principles that influence the strength of a protein/ligand complex. The present work deals with in-silico molecular docking studies of some heterocyclic compounds such as benzoxazole, benzimidazole, imidazole and tetrazole against the EGFR tyrosine kinase receptor.
Methodology: Molecular docking studies of some heterocyclic compounds such as benzoxazole, benzimidazole, imidazole and tetrazole against the EGFR tyrosine kinase receptor using Schrodinger LLC (Maestro 9.2) software.

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Results: Our in silico observations reveal that, all the selected heterocyclic compounds (1-8) show good binding interaction and good docking score against selected target enzyme. Out of eight compounds selected for the study two compounds compound 3 and 7 shows higher glide score. Compound 3 binded to ASP855 with a docking score of −11.20 kcal/mol. Compound 7 binded to ASP855 with a docking score of −11.56kcal/mol.

Conclusion: Docking results revealed that compounds (1-8) interact with EGFR kinase receptor active site. Among the compounds, compound 7 has shown the highest glide score of -11.56 kcal/mol.

1. INTRODUCTION

Enzyme-substrate, drug-protien, and drug-nucleic acid interactions are examples of elemental biomolecular interactions all aided by molecular recognition. A greater perceptive of the underlying principles that manage the nature of ligand-protein connections could lead to the development of a theoretical framework for mapping the required effectiveness and explicitness of effective drug give rise for a certain therapeutic target. Structure data for the target of interest, as well as a method for evaluating prospective ligands, are required for experimental use of this knowledge. As a result, there are a variety of computational docking approaches to choose [1]. The epidermal growth factor receptor (EGFR) is one of four members of a family of cell surface receptor tyrosine kinases that includes EGFR (HER1, HER2, HER3, and HER4). In numerous tissue types, the EGFR signalling cascade is important for cell proliferation, differentiation, and migration. Many tyrosine kinases function as integral transmembrane receptors, converting extracellular signals into intracellular responses. EGFR ligands are thought to play a direct role in tumour formation and progression, according to several lines of evidence. Changes in receptor tyrosine kinase (TK) protein expression and activity have been linked to the progression of several malignancies. As a result, the EGFR tyrosine kinase is a promising therapeutic target in cancers that produce EGFR or have a mutant or amplified EGFR gene. In EGFR-overexpressing cells, the "knocking out" of EGFR-TK activity and the DNA damage caused by the alkylating species are expected to result in a long-term antiproliferative effect [2]. Benzimidazole and other heterocyclic compounds have been developed as selective and efficient EGFR inhibitors [3], Benzoxazole [4], Imidazole [5] and Tetrazole [6]. We created Benzimidazole, Benzoxazole, Imidazole, and Tetrazole derivatives as possible kinase inhibitors for EGFR kinase based on our prior research [7].

Heterocyclic compounds have a wide range of physical, chemical, and biological characteristics. Antitumor, antibacterial, antiprotozoal, and antimicrobial properties have been found for benzimidazole, as well as suppression of the angiopoietin receptor TIE-2 and the tyrosine kinase receptor VEGFR-2 (vascular endothelial growth factor receptor-2) [8]. Benzoxazole compounds possess various biological activities for example, antimicrobial, antihistaminic, antiparasitics, herbicidal, antiallergic and antihelmintic acticivities [9]. Medicinal properties of imidazole include anticancer, anti-inflammatory, antiviral, antitubercular, antibacterial, antifungal, antidiabetic, antimalarial β-lactamase inhibitors, 20- HETE synthase inhibitors, carboxypeptidase inhibitors, hemeoxygenase inhibitors and antiaging agents [10]. Tetrazole and its derivatives are evaluated for various biological activities such as antibacterial, antifungal, antiallergic, antiviral, anticonvulsant, anti-inflammatory properties[11].

Here we would like to report the molecular docking study of some heterocyclic compounds such as benzoxazoles, benzimidazoles, imidazoles and tetrazoles (Fig.1) against EGFR kinase receptor.

2. MATERIALS AND METHODS

2.1 Docking Protocol

The RCSB Protein Data Bank was used to obtain the protein-ligand complex (PDB: 1xkk). The protein structure of EGFR kinase was created using the Schrodinger's Protein Preparation wizard. The OPLS-2005 force field was used to minimize protein. Sitemap was used to find the binding locations. The Glide programme was used to perform receptor docking against the receptor utilizing the ligands. In the receptor grid

generation, the scaling factor for protein van der Waals radii was 1.0. The centroid for generating grid files for docking was the ligands in the active sites. The Glide programme provided the default grid size. For all docking computations, the Glide extra precision mode was used. The lower the glide score, the better the binding.

2.2 Druglikeness, Pharmacokinetics, and Toxicity Analysis

Druglikeness and pharmacokinetics are two concepts that have been widely exploited in the pharmaceutical toward off side effects induced by tiny compounds. In the current work, the best hit compounds from molecular interaction studies were further analyzed for physiochemical

attributes using the Molinspiration tool to find a lead contender. Swiss ADME[12] was used to investigate pharmacokinetics and medicinal chemistry friendliness factors. The'mcule-Toxicity checker' was used to identify potentially hazardous substructures in the selected compounds.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking Study

Schrodinger LLC (Maestro v 9.2) was used to dock the proposed chemicals into the EGFR tyrosine kinase. The Protien data bank provided the crystal structure of the enzyme with laptinip: pdb code: 1xkk.
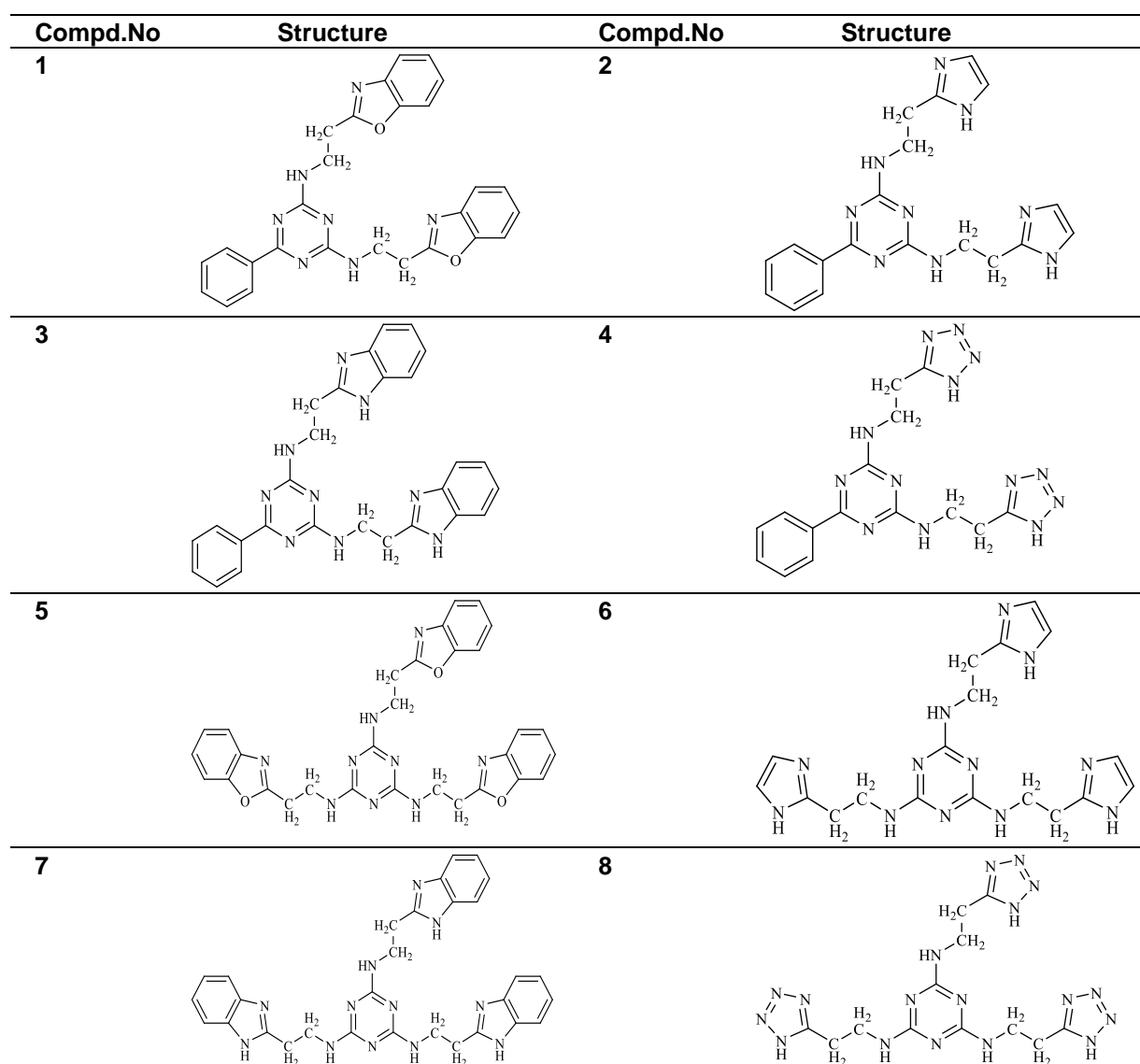

Fig. 1. Structure of the heterocyclic compounds

To investigate the binding interactions of the reference ligand, redocking of lapatinib in the ATP binding domain of the kinase activity was performed, revealing that four amino acids are involved in the interaction: ASP855, ASP800, MET793 and CYS775.

The enzyme EGFR kinase's amino acid residue ASP855-O forms a hydrogen bond with the NH of compound 1 at a hydrogen bond distance of 1.818Ao(Fig.2). The amino acid residue ASP855-O of the receptor EGFR kinase is involved in three hydrogen bond interactions with the ligand 2 with the bond distance of 1.699, 1.596 and 2.200 Ao. The amino acid residue ASP855-C=O of the enzyme EGFR kinase is involved in hydrogen bond interactions with NH of imidazole ring (3) with a bond distance of 2.113, 1.971 and 1.790Ao(Fig.3)

The nitrogen atom of the benzoxazole ring 5 made hydrogen bond interaction with NH of LYS745 of the enzyme EGFR kinase with a bond distance of 2.248Ao and forms another hydrogen bond with the C=O of ASP855 amino acid residue with the bond distance of 1.675Ao (Fig.4).

The ligand compound 7 is involved two hydrogen bond interactions with the amino acid residue ASP855-O of the receptor EGFR kinase with a distance of 2.100 and 1.575 Ao (Fig.5). With a bond length of 1.950Ao, the amino acid residue ASP855-O of the receptor EGFR kinase is bound to the NH of compound 6. The Table (Table 1) shows the glide score, glide energy, hydrogen bond donor and acceptor, and distances.

Log P, Molecular weight, Topological polar surface area (TPSA), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), and number of rotatable bonds were used to calculate druglikeness(Table 2). A bioactive molecule with log P5; MW500 Da; HBAs10 and HBDs5 exhibits smooth membrane permeability, good oral bioavailability and high gastrointestinal absorption in the human gut, as per Lipinski's Rule of Five [13]. Veber's rule states that a molecule has high oral bioavailability if its TPSA140 and total number of rotatable bonds are both less than ten [14]. The 'GSK 4/400 rule' states that a drug's tendency to be hazardous increases when its log P>4 and MW>400 Da [15]. The physiochemical properties of the eight compounds (1-8) were found to be in perfect accordance with RO5 and Veber's rules, suggesting that they have significant drug-like qualities, as well as the GSK 4/400 rule, indicating that they are nontoxic.

Table 1. Docking study results of the Compounds with Protein EGFR Tyrosine Kinase

Compd. Code	Glide score	Glide energy (kcal/mol)	Donor	Acceptor	Distance A°
1	-10.06	-68.42	NH	C=O (ASP855)	1.818
2	-9.44	-61.62	a) NH	C=O (ASP855)	1.699
			b)NH	C=O (ASP855)	1.596
			c)NH	C=O (ASP855)	2.200
3	-11.20	-72.82	a) NH	C=O (ASP855)	2.113
			b)NH	C=O (ASP855)	1.971
			c)NH	C=O (ASP855)	1.790
4	-9.09	-62.89	a) NH	C=O (ASP855)	1.771
			b)NH	C=O (ASP855)	1.633
			c)N	NH (ASP855)	2.435
			d)NH	C=O (CYS775)	2.264
5	-10.05	-78.35	a) N	NH (LYS745)	2.248
			b)NH	C=O (ASP855)	1.675
6	-9.65	-63.53	NH	C=O (ASP855)	1.950
7	-11.56	-77.92	a)NH(Ben)	C=O (ASP855)	2.100
			b)NH	C=O (ASP855)	1.575
8	-9.46	-63.71	a) NH	C=O (ASP855)	2.560
			b)NH	C=O (ASP855)	1.889
			c)NH	C=O (ASP855)	1.953
			d)NH	C=O(MET793)	2.147
			e)NH	C=O(MET793)	2.053

Table 2. Physiochemical characteristics of the compounds

| Compd. No	Mol.wt	LogP	TPSA	H-ond donors	H-Aceptor	Rotatable bonds
1	477.52	4.72	114.79	2	7	9
2	375.43	2.14	120.09	4	5	9
3	475.55	2.34	120.09	4	5	9
4	379.38	0.98	171.65	4	9	9
5	561.59	4.14	152.85	3	9	12
6	408.46	1.67	160.80	6	6	12
7	558.64	1.98	160.80	6	6	12
8	414.39	-0.13	238.14	6	12	12

Table 3. Pharmacokinetic characteristics of the compound

Compd. No	Water Solubility	Pharmacokinetic analysis			CYP450 inhibition				
		GIAb	BBBp	P-gps	CYP1A2	CYP2C19	CYP2C9	CY2D6	CYP3AI
1	Insoluble	high	No	Ye S	No	No			
2	Soluble	high	No	Ye S	Yes	Yes	Yes	Yes	Yes
3	Insoluble	high	No	Ye S	Yes	Yes	Yes	Yes	Yes
4	Soluble	Low	No	Ye S	Yes	No	No	No	Yes
5	Insoluble	Low	No	No	No	Yes	Yes	No	Yes
6	Soluble	Low	No	Ye S	Yes	Yes	No	No	Yes
7	Insoluble	Low	No	No	Yes	Yes	No	No	Yes
8	Moderately Soluble	Low	No	Yes	No	No	No	No	Yes

Table 4. Medicinal chemistry friendliness and Toxicity substructure analysis of the compounds

Compd.No	PAIN	Brenk	Toxicity substructure
1	No	No	No
2	No	No	No
3	No	No	No
4	No	No	No
5	No	No	No
6	No	No	No
7	No	No	No
8	No	No	No

Fig. 2. Interaction of Compound 1 with the ATP binding site of the EGFR TK
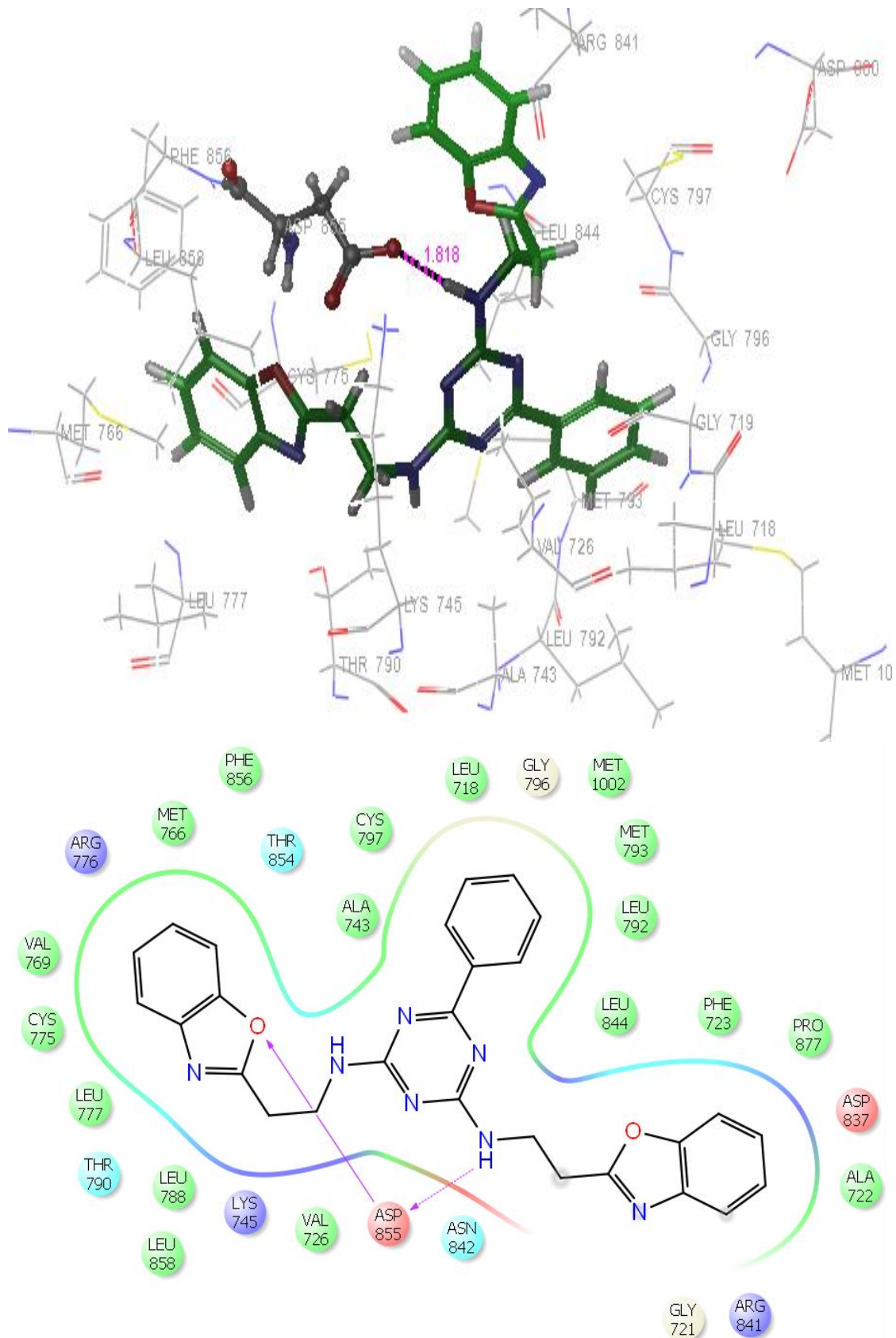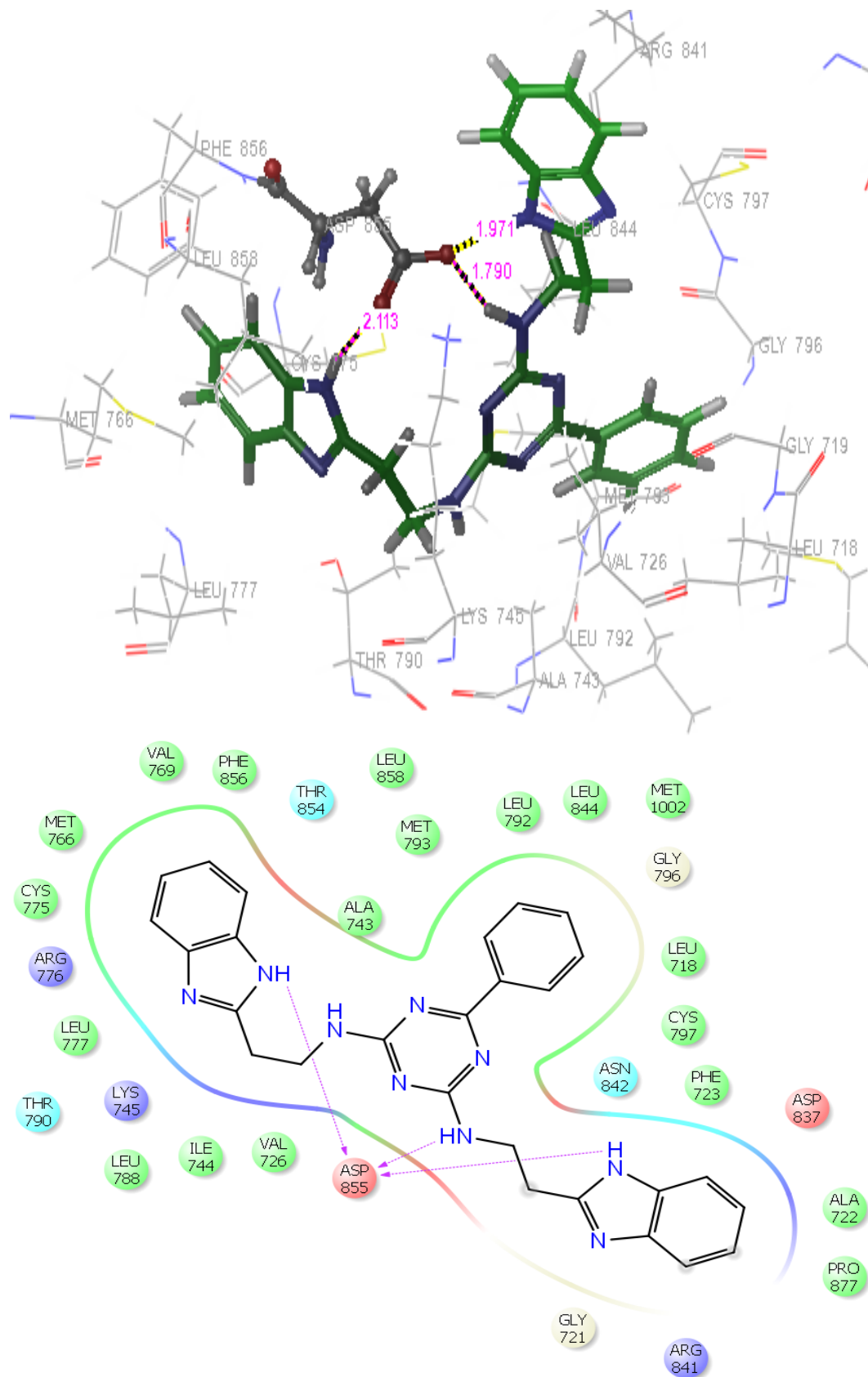
Fig. 3. Interaction of Compound 3 with the ATP binding site of the EGFR TK

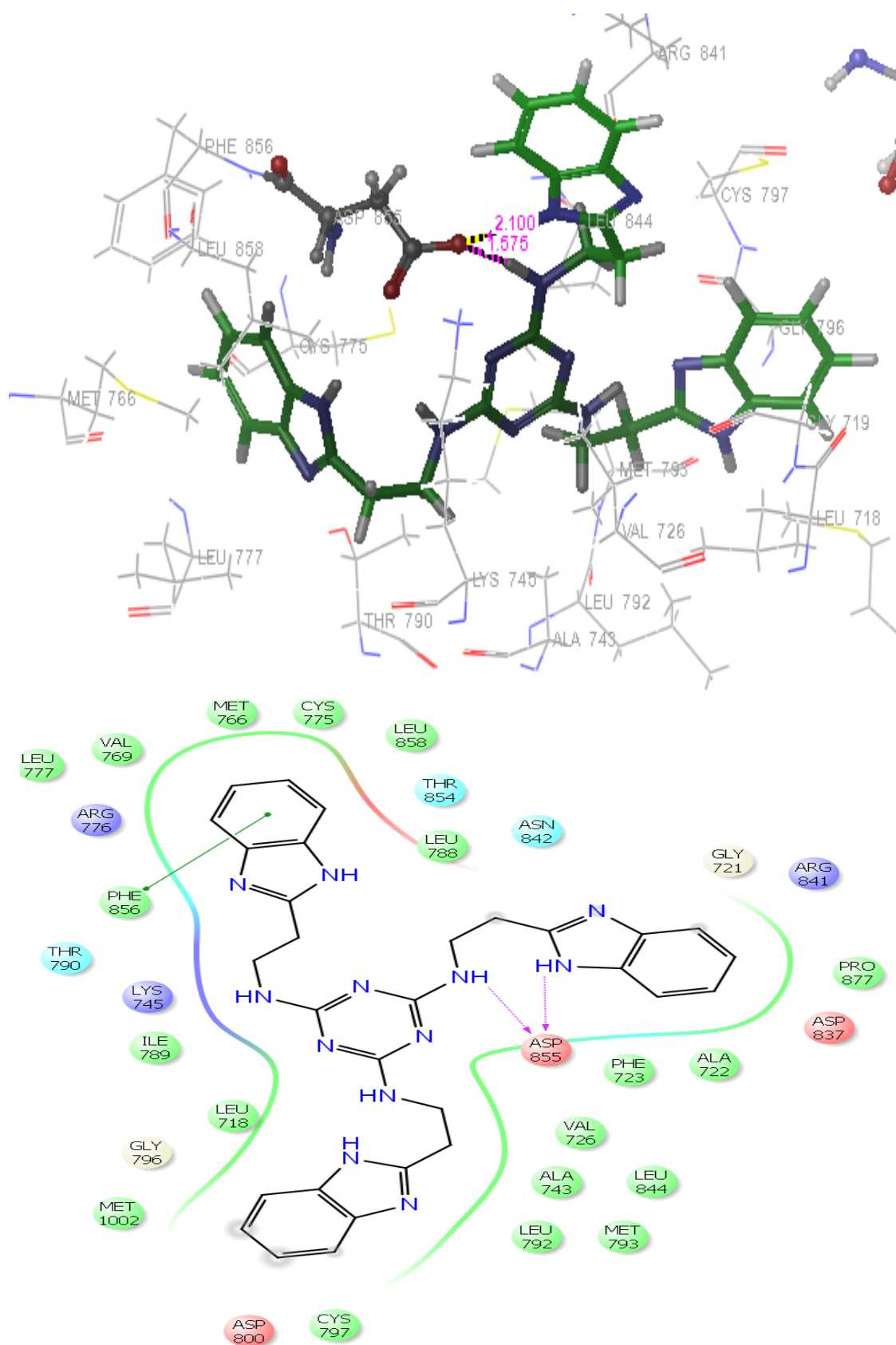**Fig. 4. Interaction of Compound 5 with the ATP binding site of the EGFR TK**

Fig. 5. Interaction of compound 7 with the ATP binding site of the EGFR TK

The most of therapeutic failures in the pharmaceutical industry are due to a lack of proper ADME analysis. Table 3 shows some essential pharmacokinetic features such as water solubility, blood brain barrier permeability, P-glycoprotein substrate, gastrointestinal absorption and inhibition of CYP450 enzymes. Water solubility testing found that all of the compounds in concern were soluble, with the exception of compounds 1, 3, 5, and 7. Compounds 1-3 had a high GI absorption rate, while compounds 4-8 had a low absorption rate. HCV and HIV infection, may penetrate the blood-brain barrier, causing neuroinflammation, according to recent research findings [16]. None of the chemicals chosen pass across the BBB. P-gp substrate analysis suggests that all of the selected hits, with the exception of compounds 6 and 7, are non-substrates. P-gp plays a key function in restricting drug uptake in cells, resulting in therapeutic failure due to lower than predicted drug concentrations [17,18]. The interaction of small molecules with several Cytochrome P450 isoforms, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, is a major determinant in drug clearance via metabolic biotransformation. The buildup of metabolites/drugs caused by the inhibition of these isoenzymes is a major cause of pharmacokinetics-related drug-drug interactions[19]. Four Cytochrome P450 isoforms are non-inhibited by compound 8. Compound 3 inhibits all five Cytochrome P450 isomers, while compound 4 inhibits three and compounds 1, 5, 6, and 7 inhibit two isoforms each. 1 isoform is inhibited by compound 2. (CYP2C9).

Furthermore, structural alarms (PAINS and Brenk) and leadlikeness were anticipated as medicinal chemistry friendliness parameters. Compounds having structural warnings must be identified as part of the drug development screening process. PAIN is a class of toxicophores that have been proven to interfere with biological testing, interact with DNA/proteins, and induce DNA/protein damage [20]. Brenkis is another structural alarm that warns of potentially dangerous, biologically unstable, and chemically reactive components [21]. PAINS and Brenkalerts were not identified in any of the compounds (1-8) tested. According to Teague's [22] leadlikeness criterion, all of the compounds admitted leadlikeness and were thus appropriate for further improvement. They were also put through the mcule-Toxicity checker, which revealed that none of the compounds had any potentially hazardous substructure. Table 4 shows the projected outcomes in greater detail.

4. CONCLUSION

In the present study molecular docking studies of a new benzimidazole, benzoxazole, imidazole and tetrazole against EGFR tyrosine kinase has been described. All the compounds were docked with EGFR tyrosine kinase receptor. The glide score of the compounds are found to be -10.06(1), -9.44(2), -11.20(3), -9.09 (4), -10.05(5), -9.65(6), -11.56(7), and -9.46(8). It is worthy to note that the compound 7 has shown the highest glide score of -11.56kcal/mol. The binding site and hydrogen bonding interactions for each drug varied, as shown in the table (Table 1). It's important to note that, while all of the compounds have the same fundamental structure, the degree of interaction and binding sites are found to be varied.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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