Bulletin of Pharmaceutical Research 2024;14(3):191

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

DOI: 10.21276/bpr.2024.14.3.3

RESEARCH PAPER

DISCOVERY OF NOVEL 4-AMINOPTERIDINE DERIVATIVES AS EGFR INHIBITORS: AN *IN SILICO* APPROACH

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Received: Dec 13, 2023 / Revised: Nov 08, 2024 / Accepted: Nov 09, 2024

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of the receptor tyrosine kinase that plays crucial role in many different cells signaling pathways. EGFR overexpression leads to the growth of cancerous cells. As a result, EGFR is considered as one of the key targets for cancer therapy. In this study, ten novel 4-amino pteridine derivatives were designed and *in silico* studies were performed to find out novel hits as EGFR inhibitors. The *in silico* methods includes molecular docking studies, druglikeness screening, *in silico* toxicity screening and bioactivity prediction. All the designed compounds had druglikeness properties and showed no violations as per the Lipinski rule. In docking studies, three compounds QZ7, QZ8 and QZ10 were found which had higher binding affinity as compared to standard drug, lapatinib. Bioactivity prediction of designed compounds suggested that all the compounds may act through kinase inhibition. Out of the ten compounds, three compounds QZ7, QZ8 and QZ10 were found with good potential to be explored as EGFR inhibitors.

Key words: EGFR inhibitor, 4-Aminopteridine derivative, Cancer, *In silico* study, Docking.

INTRODUCTION

In multicellular organisms, a balance between intercellular and intracellular communication is crucial for normal and healthy living. The passage of information between cells *via* a number of signaling pathways is crucial for different biological activities. This signaling pathways includes One of the key signaling pathways involved in numerous cellular processes such cell growth, multiplication, and death are receptor tyrosine kinases (RTKs) [1, 2]. The importance of RTKs in cancer was made clear by recent developments in the field of oncology. The development of molecular biology makes it easier to pinpoint the precise mechanism of RTK-mediated oncogenic activation [3, 4]. RTKs contain cytoplasmic domain receptors in addition to cell surface receptors. Normal cellular tyrosine kinase phosphorylation levels are strictly regulated. Multiple mechanisms exist in cancer that activate RTKs in an oncogenic manner. These aberrant actions improved signal generation, RTK oncogene fusions, gene amplifications, and oncogenic mutations. RTKs can become oncogenic activated by overexpressing ligands or adaptor proteins, and by mutating signaling pathways [5, 6].

RTKs are divided into several subfamilies, including the insulin-like growth factor receptor, fibroblast growth factor receptor, vascular endothelial growth factor receptor, and epidermal growth factor receptor. RTKs from different subfamilies are explored as cancer therapeutic targets [7]. The epidermal growth

factor receptor (EGFR) is a member of the ErbB family of the receptor tyrosine kinase that plays crucial role in many different cell signaling pathways. Four structurally conserved members make up these families: EGFR, ErB-2 (HER-2), ErB-3 (HER-3), and ErB-4 (HER-4) [8, 9]. Specific ligands activated these receptors by binding to them. The extracellular portion of the receptor causes homo- and/or heterodimerization of the receptors upon interaction of the growth factors, which activates the tyrosine kinase domain to phosphorylate at its C-terminal tail. The downstream signaling pathways are activated by these processes [10, 11]. EGFR overexpression leads to the growth of cancerous cells. The majority of cancer-related deaths are caused by overexpression of EGFR, which is seen in about 60% of patients with non-small cell lung cancer. As a result, EGFR is considered as one of the key targets for cancer therapy. Currently, EGFR-mutated tumors are treated clinically with monoclonal antibodies (MAbs), antisense oligonucleotides, antibody-based immune-conjugates, and the small molecules. These tiny compounds compete with ATP at the EGFR's TK domain. Examples of small drugs that target the EGFR include gefitinib, erlotinib, lapatinib, etc. Apart from this, attempt have been also made to discover natural products that can target EGFR kinase domain [12-14].

Development of novel drug is very expensive and time-consuming process. Receptor based virtual screening is powerful and inexpensive technique to identify novel lead compound in drug discovery process. This technique required knowledge of 3D structure of protein and its binding site [15-19]. Pteridines are aromatic chemicals created when the rings of pyrimidine and pyrazine combine. Pteridines are produced by many living things, and they are used in enzymatic and pigment processes. immune system activation molecules, also known as cofactors. In order to investigate their therapeutic potential, a vast array of pteridine derivatives have been synthesized as a result of this diversity of biological roles. One of the most researched and developed therapeutic potentials of pteridine-based drugs is their anticancer action, for which various molecular targets have been identified. In the literature, there are so may evidences which suggest the potential of pteridine derivatives to target EGFR receptors. In this research work, ten novel 4-amino pteridine derivatives were designed and *in silico* studies were performed to find novel 4-amino pteridine derivatives as EGFR inhibitors [20].

MATERIAL AND METHODS

Protein preparation

The three-dimensional (3D) structure of human EGFR kinase domain complexed with lapatinib was retrieved from Protein Database Bank (PDB id: 1XKK). Protein structure was evaluated by Ramachandran plot using RAMPAGE. The Ramachandran plot states that 265 (95.7%) residues of the predicted model of EGFR were in favored region while 11 (4%) were in allowed region and 1 (0.4%) were in outlier region (**Figure 1**). The preparation of protein includes addition of hydrogen bond and charges as well water molecules were removed using AutoDock4. Finally, 3D structure of protein saved in PDB format and was used for docking.

Ligand preparation

The 2D structures of all designed compounds were drawn using ChemBioDraw Ultra 12.0 (**Figure 2**). The geometry of structures was corrected and energy minimized using ChemBio3D Ultra 12.0. Then, structure were saved in mol format and were used for docking.

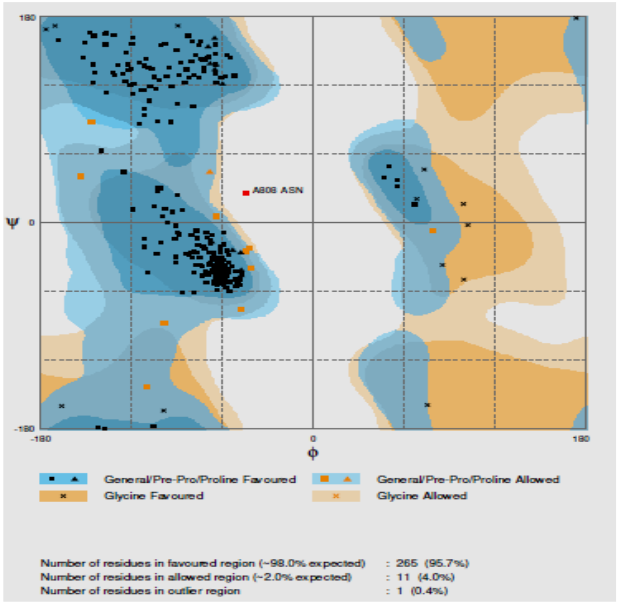

Fig. 1. Ramachandran plot of predicted model of protein 1XKK presented dihedral angles Φ against Ψ. Summary of the residues is at the bottom of the image.

Molecular docking

The molecular docking calculations were performed using iGEMDOCK and flexible ligand

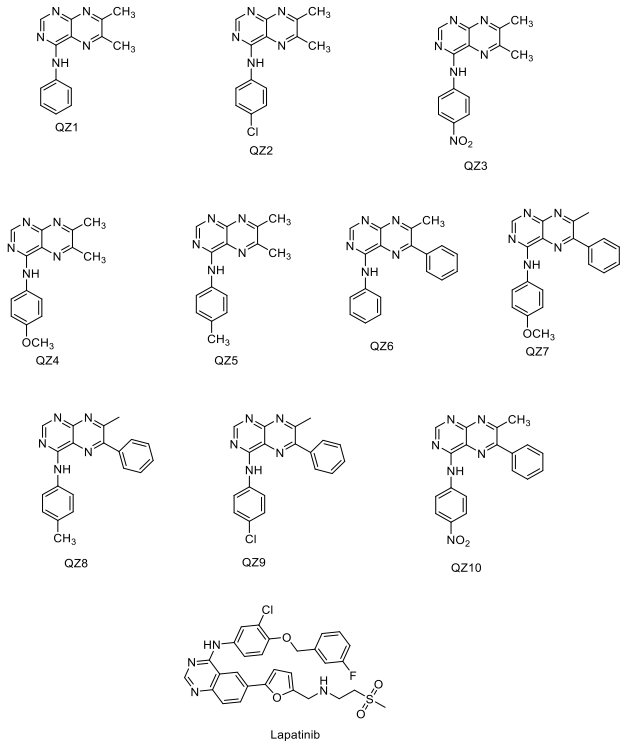

Fig. 2. Structures of designed compounds and standard drug - Lapatinib

docking was applied to predict inhibitors targeting EGFR kinase domain. The binding sites of the targets were prepared and the energy minimized compound was imported. From the docking wizard ligands were selected and the scoring function used was iGEMDOCK score. The binding site of the target was selected within 8Å root mean square deviation (RMSD) to improve docking accuracy. Visualization of protein-ligand interactions were carried out using PyMol. Lapatinib was used as reference drug to select the hits. The standard docking mode was selected which consisted of 70 generations per ligand and the population size of 200 random individuals. The scores were compared with standard drugs - lapatinib erlotinib and gefitinib. The hits which get higher score than standard drugs were again rescored using accurate docking mode of iGEMDOCK which consisted of 80 generations per ligand and the population size of 800 random individuals and validated for drug-likeliness using Lipinski rule of five.

In silico ADMET studies

The compound may fail in pre-clinical studies if they don't have required pharmacological properties to be considered as drug molecule. Compounds ADMET profile plays crucial role in development of drug. Therefore, compounds have to pass multiple filters to be considered as novel drug. *In silico* toxicity studies are faster and reduce the amount of animal experiments. The *in silico* toxicity studies were performed using ProTox-II online tool. The properties such as organ toxicity, carcinogenicity, mutagenicity, cytotoxicity, and toxicity class were predicated.

In silico bioactivity analysis

Using the tool Molinspiration Cheminformatics server, the bioactivity score of screened flavonoids was assessed. This method uses data from extensive chemical databases to find potential novel medication candidates. In order to compare active and inactive compounds, the tool first examines a training set of active molecules using sophisticated statistical model. Score is produced in accordance with this action. Probability of active chemical is high with high score.

RESULTS AND DISCUSSION

Molecular docking studies

Molecular docking studies were performed using iGEMDOCK. Docking and post analysis were done using iGEMDOCK. The designed molecules were screened based on the docking score of standard drug - lapatinib. The docking score of designed compounds were found in the range between -83.82 to -114.11. The docking score of all designed compounds and their interactions are shown in **Table 1**. The standard drug lapatinib had docking score of -102.92. In comparison with standard, three designed ligand QZ7, QZ8 and QZ10 had lower docking score i.e. higher binding affinity. The docking score of QZ7, QZ8 and QZ10 were -102.96, -103.13 and -114.11, respectively. The active residues found in the binding pocket were ASP855, VAL726, LEU718, GLY719, VAL726, LYS745, GLY796, LEU844, THR854 and ASP855. All the designed ligands had interaction with active residues within the binding site only which denotes accuracy of docking protocol. The designed compound QZ7 formed Vanderwall's interactions with LYS745, LEU844, THR854 and ASP855. The designed compound QZ8 had one H-bond interaction with VAL726 and Vanderwall's interactions with LYS745, LEU844, THR854 and ASP855. The designed compound QZ10 had one H-bond interaction with VAL726 and Vanderwall's interactions with LYS745, LEU844, THR854 and ASP855. The standard drug, lapatinib had Vanderwall's interactions with LEU718, GLY719, VAL726, LYS745, GLY796, LEU844, THR854, ASP855 (**Figure 3**).

Table 1. Docking score and interacting residues of designed compounds

Compound	Docking score	H-bond	VdW	Interacting residues
QZ1	-83.92	-70.26	-13.66	VAL726, LYS745, LEU844, THR854, ASP855
QZ2	-83.82	-70.26	-13.56	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ3	-101.38	-87.98	-13.39	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ4	-88.31	-74.84	-13.46	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ5	-96.84	-83.02	-13.82	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ6	-94.57	-91.07	-3.5	VAL726, LYS745, LEU844, THR854, ASP855
QZ7	-102.96	-97.42	-5.54	VAL726, LYS745, LEU844, THR854, ASP855
QZ8	-103.13	-89.13	-14	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ9	-99.68	-85.68	-14	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
QZ10	-114.11	-97.23	-17.12	ASP855, VAL726, LYS745, LEU844, THR854, ASP855
Lapatinib	-102. 92	-88.82	-14.1	LEU718, GLY719, VAL726, LYS745, GLY796, LEU844, THR854, ASP855

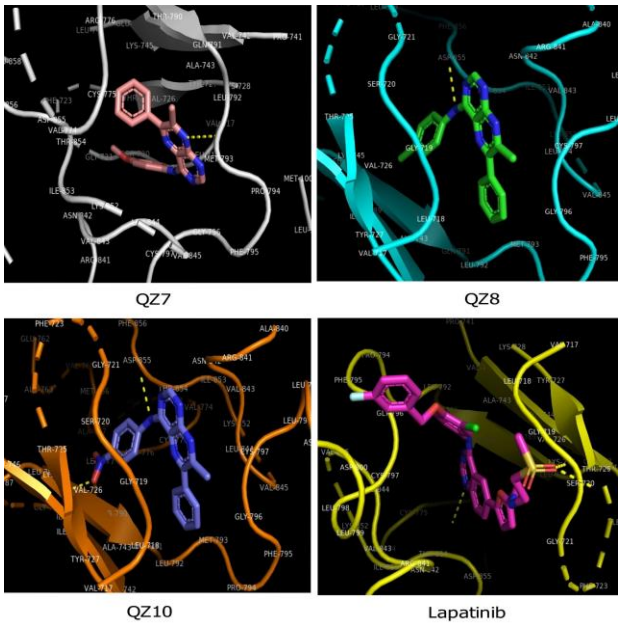

Fig. 3. Molecular interactions of compound QZ7, QZ8, QZ10 and standard drug lapatinib with various amino acids of protein EGFR

In silico druglikeness and toxicity studies

If a molecule already in existence shares chemical characteristics with well-known medications, it is said to be drug-like. Lipinski's Rule is the primary criterion for evaluating a chemical compound's drug similarity. Determining whether a chemical molecule possesses specific molecular characteristics that would make it an orally effective drug in humans or not, is a crucial criteria in drug likeness analysis. The rule shows aspects of a drug's pharmacokinetics, such as its absorption, distribution, metabolism, excretion, and toxicity, that are essential. The majority of the time when estimating drug similarity, Lipinski's Rule of Five and ADMET characteristics are taken into account. The rule of 5 states that poor absorption or penetration is more likely in the context of drug discovery when there are more than five H-bond donors, ten H-bond acceptors, a molecular weight larger than 500, and a computed Log P (CLog P) greater than five. All the selected compounds pass the Lipinski's rule and showed no violations (**Table 2**). To identify a chemical's adverse effects on human, animals, plants, or the environment, its toxicity must be determined. It is also a crucial stage in the creation of drugs.

In order to test for toxicity, animal models have been employed for a long time. But there is time, ethical, and economical constraints on *in vivo* animal testing. Therefore, computational methods are thought to be useful for assessing the toxicity of substances. *In silico* toxicology is a branch of toxicity assessment that analyses, simulates, visualizes, or predicts the toxicity of compounds using the computational methods.

To forecast toxicity, prioritize compounds, direct toxicity studies, and reduce late-stage drug design errors, in silico toxicology seeks to

supplement existing toxicity tests. The *in silico* toxicity predations suggest that designed compound may have minimal or tolerable toxicity (**Table 3**).

Table 2. Drug likeness screening of designed compounds

Compound code	MW	LogP	HBA	HBD	Nrotb	nviolations
QZ1	251.29	2.85	**8**	1	2	**0**
QZ2	285.74	3.36	5	1	2	0
QZ3	296.29	2.64	8	1	3	0
QZ4	281.32	2.74	6	1	3	0
QZ5	265.32	3.13	5	1	2	0
QZ6	313.36	4.14	5	1	3	0
QZ7	308.74	4.19	6	1	4	0
QZ8	327.29	4.58	5	1	3	0
QZ9	347.81	4.81	5	1	3	0
QZ10	358.36	4.09	8	1	4	0
STD	581.07	6.16	8	2	11	2

Table 3. *In silico* toxicity studies of designed compounds

Compound code	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Cytotoxicity	LD_{50} (mg/kg)	Toxicity class
QZ1	**Active**	Inactive	Inactive	Inactive	1800	**3**
QZ2	**Active**	Inactive	Inactive	Inactive	1800	**3**
QZ3	**Active**	**Active**	Inactive	Inactive	1800	**3**
QZ4	**Active**	Inactive	Inactive	Inactive	1800	**3**
QZ5	Inactive	Inactive	Inactive	Inactive	1800	**3**
QZ6	**Active**	Inactive	Inactive	Inactive	1800	3
QZ7	**Active**	Inactive	Inactive	Inactive	1800	3
QZ8	**Active**	Inactive	Inactive	Inactive	1800	3
QZ9	**Active**	Inactive	Inactive	Inactive	1800	3
QZ10	**Active**	Inactive	Inactive	Inactive	1800	**3**
STD	**Active**	Inactive	Active	Inactive	1500	**4**

In silico bioactivity analysis

Additionally, the programme Molinspiration was used to evaluate the bioactivity score of the chosen medicines. In the Molinspiration tool, the miscreen engine analyses a training set of active structures (in extreme cases, just one active molecule is enough to develop a useful model) and compares them against inactive molecules using advanced Bayesian statistics. The likelihood of being active is higher for molecules with the highest activity score. Large collections of molecules (more than 100,000 molecules) can be screened using this type of in silico screening in about an hour. Screening models for four significant pharmacological classes—GPCR ligands, ion channel blockers, kinase inhibitors, and nuclear receptor ligands—were created based on the methods previously outlined. The Bioactivity prediction of screened compounds mentioned in **Table 4**. The results of *in silico* bioactivity prediction shows that screened flavonoids may act through kinase inhibition.

Table 4. Bioactivity prediction of designed compounds

Compound code	GPCR	ICM	KI	NRL	PI	EI
QZ1	0.11	0.13	**0.39**	-1.05	-0.47	**0.29**
QZ2	0.14	0.11	**0.39**	-0.97	-0.46	**0.25**
QZ3	0.02	0.05	**0.26**	-0.90	-0.45	0.15
QZ4	0.11	0.02	**0.38**	-0.88	-0.41	**0.22**
QZ5	0.10	0.05	**0.37**	-0.97	-0.45	**0.24**
QZ6	0.14	-0.01	**0.53**	-0.46	-0.39	0.19
QZ7	0.08	-0.09	**0.45**	-0.45	-0.41	0.13
QZ8	0.10	-0.07	**0.47**	-0.47	-0.40	0.14
QZ9	0.13	-0.02	**0.49**	-0.47	-0.41	0.15
QZ10	-0.02	-0.06	**0.32**	-0.51	-0.46	0.07
STD	-0.04	-0.52	**0.36**	-0.35	-0.21	-0.08

CONCLUSION

EGFR overexpression leads to the growth of cancerous cells. The majority of cancer-related deaths are caused by overexpression of EGFR, which is seen in about 60% of patients with non-small cell lung cancer. As a result, EGFR is considered as one of the key targets for cancer therapy. 4-Amino pteridine inhibitors are known to target various tyrosine kinase receptors including EGFR. Although various drugs have been developed to target EGFR mutated cancer; still there is need to explore new inhibitors which can overcome various limitations current EGFR inhibitors. We had designed ten novel 4-aminpteridine derivatives to target EGFR using different *in silico* methods.

Compounds were screened based on molecular docking studies, druglikeness screening, toxicity screening and *in silico* bioactivity prediction. We found three compounds QZ7, QZ8 and QZ10 that had higher binding affinity compared to standard drug lapatinib. These screened compounds also found safer in *in silico* toxicity profile. Finally, it is concluded that compound QZ7, QZ8 and QZ10 can be further explored as EGFR inhibitors.

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