# Targeted Molecular Docking and Pharmacokinetic Evaluation of Designed Pyrimidine and Pyridine Derivatives Targeting Signaling Protein EGFR Involvement in Glioblastoma

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## Abstract

*Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with tyrosine kinase activity. EGFR is frequently overexpressed and/or hyper-activated in human malignancies, including glioblastoma, leading to development of aggressive glioblastoma. The present study attempts to identify potential targets of EGFR for novel inhibitors from Nitrogen based heterocycles. A small library of 485 compounds were designed and docked into EGFR kinase domain adenosine triphosphate binding pocket. The binding mode of ligands was studied to understand the interactions between EGFR target and ligands in the series. Crystal structure of EGFR was obtained from Protein Data Bank (PDB). Molecular docking was carried out using glide and compounds with good binding affinity were selected for Prime MM-GBSA rescoring. Final list of compounds was taken for predicting pharmacokinetic properties using Qikprop. Out of 485 chalcone derivatives retrieved docking based virtual screening by different modes was used to identify compounds with good glide score compare with standard Erlotinib, selected top molecule were further subjected MMBSA evaluation to determine free binding energy. ADME and toxicity filtered were applied to screened compounds and 13 compounds were identified with good pharmacokinetic profile. Current study could identify the in-silico potential of selected novel disubstituted pyrimidine and pyridine derivatives lead as better binding affinity and stability compound to drug standard Erlotinib, Further the identified compounds displayed good pharmacokinetic and pharmacodynamic profiles. Suggesting the lead compound as target for EGFR domain and can be predicted to inhibit EGFR associated glioblastoma.*

***Keywords:*** *EGFR, molecular docking, pyrimidine compounds, various modes, Prime MM-GBSA, Qikprop.*

## 1. Introduction

Gliomas are primary brain tumors that are believed to arise from progenitor cells or neural stem cells that have genetic changes that cause tumors. They are categorized using the WHO classification of central nervous system (CNS) tumors, which places them into CNS WHO grades 1–4, which range from low to high malignancy, based on their microscopic appearance and molecular features.[1] One of the most prevalent and severe primary brain tumors in adults is glioblastoma and representing approximately 57% of all gliomas and 48% of all primary malignant central nervous system (CNS) tumours.[2,3] Primary glioblastoma develops spontaneously, whereas secondary glioblastoma, which accounts for roughly 10% of cases, occurs more frequently in younger patients and may develop from the progression of lower grade astrocytoma.[4] The primary genetic alterations linked to glioblastoma include high-level gene amplification of the proto-oncogene EGFR, TERT promoter mutation, loss of the PTEN tumor suppressor gene, ATRX mutation, and TP53 mutation.[5]

The physiological cycle of malignant tumors is controlled by receptor tyrosine kinases (ERbB family) located on the cell membranes of living organisms. RTKs play a pivotal role in management of highly malignant tumor. EGFR is a type of RTK which is reported to play significant role in controlling growth, Proliferation and different ion. EGFR mutations is key oncogene in lung cancer and Glioblastoma. Amplification of EGFR gene is the driving force in glioblastoma amplification. The heterogenicity and plasticity of tumor is enhanced during abnormal cell division. The EGFR contains an extracellular ligand binding domain along with regulatory domain and intracellular tyrosine kinase.

EGFR on binding with ligand epidermal growth factor leads to phosphorylation in intracellular domain followed by conformational change and cause signal transduction through pathway PI3K/AKT, STAT and Raf - extracellular signal regulated kinase. The end result is proliferation and apoptosis inhibition. Amplification leads to range of mutations which include Mutations, Intragenic deletion and gene fusions. Heterogenetic of tumor is due to multiple EGFR mutation with in the tumor. EGFR VIII, A289x, G598x and R108k mutations found in glioblastoma which increase tumorigenic potential in cells. An internal receptor tyrosine kinase (RTK) domain, a hydrophobic transmembrane region, an extracellular ligand-binding domain, and a C-terminal domain make up EGFR.[6] EGFR regulates the corresponding cell surface receptor and epithelial cells, which are the progenitors of all carcinomas.[7] It plays important roles in the physiology of epithelial cells and is a member of the ERbB family of receptor tyrosine kinases (RTKs). Normal development and homeostasis depend on receptor tyrosine kinases (RTKs) and the signaling pathways they produce in cells.[8] An essential protein kinase, the epidermal growth factor receptor (EGFR), is linked to a number of malignancies and, when dysregulated, can interfere with certain pathways.[9] According to an abundance of data, EGFR is over expressed in the majority of original glioblastomas and certain secondary glioblastomas. It is also indicative of more aggressive glioblastoma morphologies. There are both ligand-dependent and ligand-independent pathways that can lead to increased EGFR activation.[10] Erlotinib, Temozolomide, Sorafenib, Cedrane, Bevacizumab, Lapatinib, Afa nib, Dacomitinib and Sunitinib have been identified as EGFR inhibitors.

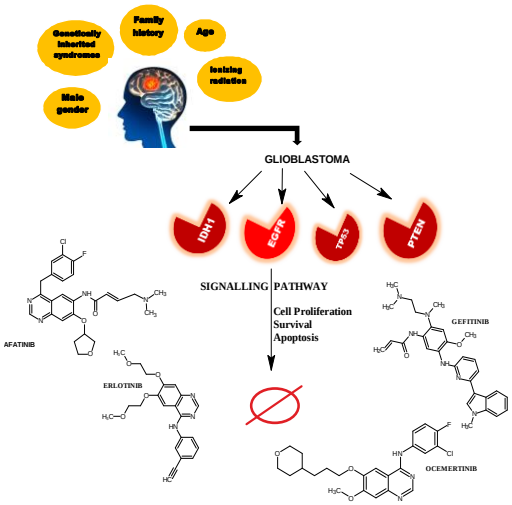


**Figure 1: Factors Influencing Glioblastoma, Role Of EGFR And Its Inhibitors**

Three generation EGFR kinase inhibitors are available. The first-generation drugs suffered from drawback of resistance in 50% of patients due to T79m mutation example Gefitinib and Erlotinib Followed by second generation drugs which prevented resistance but brought out source side effects like diarrhea and skin rashes example afatinib and docatinib. The third-generation drugs development was based on effectiveness towards T79M resistance mutation inhibition and selectively active towards EGFR. E.g. AZD9291, C01686 and C024002. The current approved EGFR inhibiters are ATP complex inhibitor targeting ATP binding site. Chemoradiotherapy and surgical excision are the usual treatments for glioblastoma.[11]. EGFR (Eepidermal growth factor receptor) inhibitors with Amino pyrimidines and Aryl-hydrazones involved in the treatment of glioblastoma such as Erlinotib, Gefitinib, Afatinib and Osimertinib fig1.

Chalcones are a class of open-chain flavonoids containing 15 carbons frame work (C6-C3-C6) with two six membered aromatic rings connected by a three carbon α, β-unsaturated carbonyl skeleton. This class of compounds have been demonstrated to have antibacterial, antifungal, anti-tubercular, cytotoxic and anti-proliferative or anticancer and antioxidant properties. The α, β-unsaturated carbonyl moiety of chalcones is the most significant structural component responsible for their qualitative activity, whilst the substituents on the two aryl rings are responsible for the intensity and range of pharmacological activities. Chalcone derivatives have been screened for their anti-inflammatory activity, chemo preventive activity, cardiovascular disease, anticancer activity, cytotoxic activity, antiproliferative activity, antimalarial activity, antiviral activity and anti-HIV activity.

Amino pyrimidines and Aryl-hydrazones such as thiosemicarbazones (TSCZs), semi carbazones (SCZs), guanyl hydrazones (GHZs) and phenyl hydrazones (PHZs) are important classes of organic compounds which have long attracted attention, owing to their remarkable biological and pharmacological properties, such as antibacterial, antiviral, anticancer, anti-inflammatory, antineoplastic, antimalarial, analgesic, and antipyretic activity. Fig2 shows structure of some marketed drugs with pyrimidine and thiosemicarbazone core. W240028, rociletinib and olmutinib are third generation EGFR inhibitors available with pyrimidine moiety.

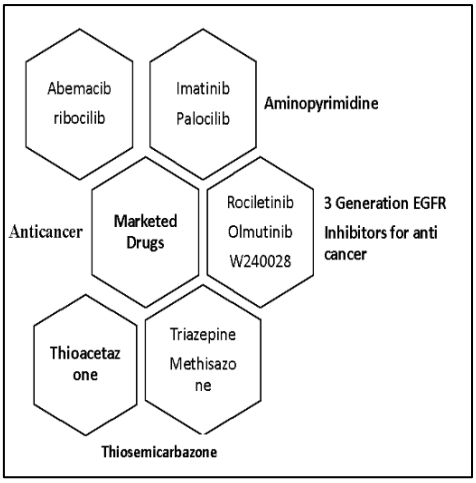


**Figure 2: A. Marketed Anti-Cancer Drugs Of Aminopyrimidine And Thiosemicarbazone. B. Reported Third Generation Aminopyrimidine Analogues With EGFR Inhibitory Activity**.

The work was aimed to screen 480 compounds (Designed based on literature of chalcones, thiosemicarbazones and aminopyrimidines) and to investigate the in-silico binding affinity by glide docking and prime energy calculations for the selected target EGFR and to predict their druglikness and pharmacokinetic properties.

To evaluate the enrichment factor of designed compounds towards EGFR crystalized graphic structure, glide and MM/GBSA calculations were applied. The current study aims at conducting docking of a dataset fragment using standard precision (SP), XP and Molecular mechanics/ generalized born surface area calculations.

## 2. Materials and method

### 2.1 Library generation and generation of 2D structures

The core generation was carried out based on the published available EGFR inhibitors [Thioacetazone, Methisazone, Erlotinib] and molecules were generally based on the side chains and core analysis. Fig 3

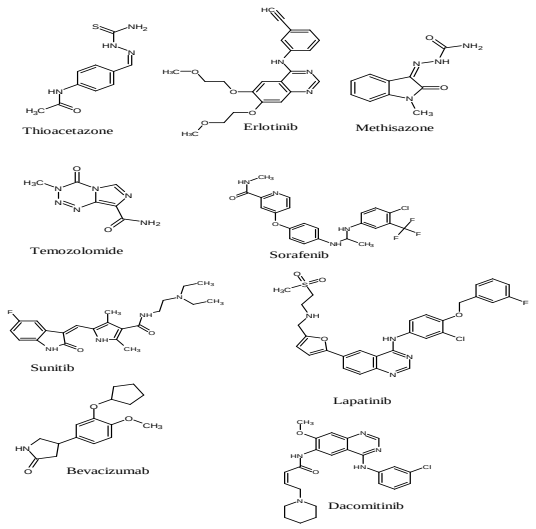


**Figure 3: Pharmacophore of approved EGFR inhibitors used for library generation**

Molecules drawn were analyzed and unwanted fragments such as heteroatom-heteroatom single bonds, Hydrolysable groups, Cytotoxic groups and interfacing scaffold were eliminated. The details are shown in Fig4.

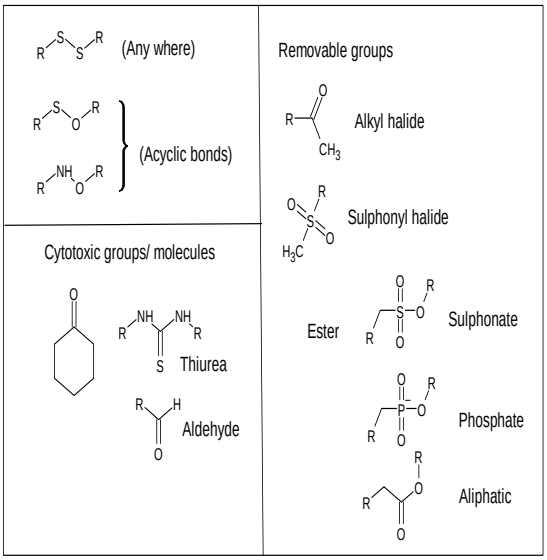


**Figure 4: Hydrolysable Groups, Cytotoxic Groups and Interfacing Scaffold to be Avoided.**

Once the structural integrity was determined. Unwanted properties were filtered by excluding using lead likeness properties like NMT 4 rings, NMT 3 fused aromatic rings, HBD > 4, NMT 4 halogens and NMT 2CF3. Screening set was finalized and Chem draw, chem sketch/ Marvin sketch was used to draw 2D structures of the selected compounds. The structural formula has been presented in Fig 5

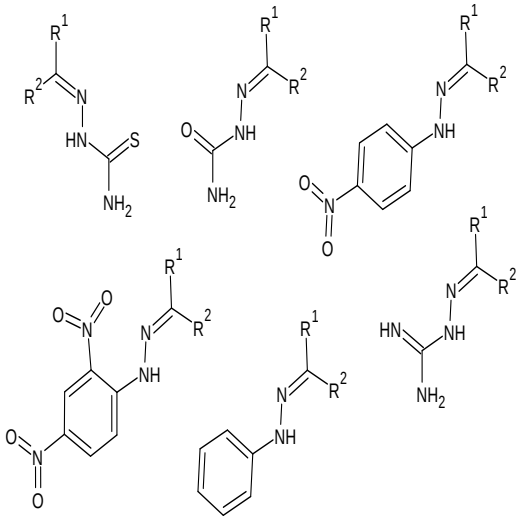

**Figure 5: Pharmacophore and substituents used for library generation**

| R1 | R2 | R1 |
|---|---|---|
| 1-(furan-2-yl) ethan-1-one | Benzaldehyde | (3Z)-4-phenyl but-3-en-2-one |
| 1-phenlypropane-1-one | Furan-2-carbaldehyde | 4-(4-methoxyphenyl)butan-2-one |
| 1-phenylpentan-1-one | (2Z)-3-phenylprop-2-enal | 1-cyclopropylethan-1-one |
| 1-(4-methoxyphenyl)ethan-1-one | 4-hydroxy-3-methoxybenzaldehyde | 1,2-diphenylethan-1-one |
| 1-(4-methylphenyl) ethan-1-one | 2-hydroxy benzaldehyde | 4-methylpentan-2-one |
| Phenyl(pyridin-2-yl) Methanone | **R3** | *N, N*-diphenylurea |
| Cyclopropyl(4-ethoxyphenyl) Methanone | Hydrazine carbothioamide | 9H-xanthen-9-one |
| Cyclobutyl(phenyl)Methanone | Hydrazine carboxamide | (4-amino phenyl) (phenyl)Methanone |
| 2,2,2-trifluoro-1-phenylethan-1-one | Hydrazine carbodiimide | 5-methyloctan-3-one |
| | Phenyl hydrazine | |

**2.2 Molecular Docking**

Glide (Schrodinger, LLC, New York N7, 2021) with operating system windows 10 was used to perform docking simulations between ligand and receptor. Glide methodology involves 3 different docking methods. HTVS (High Throughput Virtual Screening), SP (Standard precision) and XP (Extra precision) with a retaining data set of 1%, 10% and 50%. Preliminary screening of large set of compounds is carried by HTVS mode which takes approximately 2s to complete. An extensive sampling and elimination of false positive results is carried out using extra precision docking. The docking was carried on flexible mode in which automatically conformations are generated for the ligand inputted. Hierarchical filters evaluated the spatial fitting of ligand in identified active site. Further grid methodology was used to evaluate ligand - protein interaction. To add, the chem score function identified favorable interactions like hydrophobic, hydrogen, metal–chelation and inhibits (penalizes) clashes. Final scoring is done for the minimized pose using Glide score (scoring function) after non- bonded ligand receptor interaction energy is minimized. The glide score was compared with standard compounds as well as EGFR inhibitor Erlotinib, Lapatinib and Dacomitinib.

The docking scores were selected based on the fact that lower the glide score better is the affinity of compounds with EGFR. The results are tabulated (1) The free binding energy of docked complex was evaluated to determine docking pose, structural stability generalized born surface area (MM-GBSA) with prime. Prime uses OPLS3 force field and VSGB model for calculations. The results are tabulated (2)

### 2.3 Protein preparation

The crystallographic structure of EGFR with code (3POZ) was downloaded from PDB with resolution and further modulated for docking by protein preparation wizard module of Maestro 14.2. The reliability of docking protocol was validated by recording of native (TAK-285) into the binding site using HTVS, SP & XP mode. The obtained binding energy was found to be -13.0 KJ/Mol. Since the original crystal conformation over appear with best conformation the associated RMSD as (0.059A, 0.071 A, 0.068 A). The structure based virtual screening was enabled. The amino acids residues present in the active site of enzyme was identified using discovery studio. Amino acids detected were His, Asp, Arg, Glu, and Lys.

### 2.4 Ligand preparation

Ligand preparation module was used to prepare drawn 3D ligands by adding Hydrogens, Neutralizing charged groups, inclusion of ionized state and tautomers. The ligands energy minimization was carried using OPLS 2005. The prepared ligands were filtered using Lipinski rule of 5 and proceeded for virtual screening outflow using glide in Schrodinger suit maestro 14.2.

### 2.5 ADME & TOXICITY prediction

The pharmacokinetic evaluation was to predict physically significant descriptors and pharmaceutically relevant properties of absorption, distribution, metabolism, and excretion (ADME) prediction program for organic molecules. ADMET prediction helps to depict novel compounds with good oral bioavailability.

All the designed compounds were evaluated for their ADMET properties using Qikprop of Schrodinger suit maestro 14.2 version and filtered for their druglike properties. Results are tabulated (3). Molecular weight, Log p, Aqueous solubility and PSA was used to evaluate drug likeness of molecule. Drug absorption (Cacco-2 permeability), Oral absorption (MDCK permeability and BBB penetration) log B/B (Access of CNS depiction and Neurotoxicity), Herg $K^+$ channel [ Cardiac toxicity prediction], Lipinski rule for oral bioavailability. The drug likeness and pharmacokinetic criteria was accessed using Lipinski rule.

## 3. RESULTS AND DISSCUSION

The designed compounds showed varied glide score coulomb and Vander Waals energy due to different structural features. Among the screened compounds 13 compounds displayed good glide score above -10kcal/mol for targeted EGFR and also showed free binding energy Δdg binding. table (1-2).

The molecular docking studies of compounds into EGFR binding site revealed a clear preference for the binding pocket. Residues Tyr13, Ile23, Arg-41, Leu-47, Tyr-13, Leu-15, and His-16 are important for the catalytic mechanism of EGFR. Leu14, Tyr45, Leu69, and Leu98 are required for forming hydrophobic interaction with Li domain of EGFR (B loop) Val350 and

Phe357 (L2 domain A loop) Leu382, Phe412, and Ile438(L2 domain c terminal EGFR). A well-defined hydrophobic channel and blocking it is required for binding of ligand with EGFR and inhibiting it. [12,13]. The majority of the ligands displayed a greater binding affinity with the target receptor crystal structure of EGFR. The compounds were observed to interact with the active site of the target receptor with conventional, carbon hydrogen bond, electrostatic, polar and hydrophobic interactions, respectively. The selected compounds showed common hydrogen bonding with Asp776 and Lys 721, positive interaction was found with Arg B 938 & ArgA 817, negative interaction with Asp831 & Asp 776, Asn B 802 & SerA696 showed polar interaction. Compounds showed common hydrophobic interaction with ILE B942, Leu A 694, LeuA 772, Val A702, Ala 719 and Met 769. The residue interactions with EGFR are shown in the Fig: 6.

**Table 1: G-score and varied interaction of selected molecules in the active site of EGFR**

| CC | G-score | Ligand Efficiency | Type of interaction | Number of Hydrogen Bonds &residues |
|---|---|---|---|---|
| **C68** | -11.94 | -59.133 | H-B, -ve, +ve, polar, Hy-B | **4(**GLN A767, THR A 766, THR A830, ASP A776**)** |
| **C32** | -11.44 | -72.83 | H-B, -ve, +ve, polar, Hy-B | **0** |
| **C61** | -11.18 | -68.140 | H-B,-ve,+ve, polar, Hy-B | **1(**ASP A: 776**)** |
| **C69** | -10.93 | -62.47 | H-B, -ve, +ve, polar, Hy-B | **1(**ASP A: 776**)** |
| **C26** | -10.52 | -79.72 | H-B, -ve, +ve, polar, Hy-B | **1(LYS**: 721**)** |
| **C74** | -10.71 | -75.05 | H-B, -ve, +ve, polar, Hy-B | **0** |
| **C35** | -10.18 | -80.40 | H-B, -ve, +ve, polar, Hy-B | **1(**ASP A: 776**)** |
| **A36** | -9.9 | -57.32 | H-B, -ve, +ve, polar, Hy-B | **1(**ASP A: 776**)** |
| **A31** | -9.7 | -51.57 | H-B, -ve, +ve, polar, Hy-B | **1(LYS**: 721**)** |
| **A33** | -9.3 | -57.85 | H-B, -ve, +ve, polar, Hy-B | 0 |
| **STD** | -9.5 | -55.65 | H-B, -ve, +ve, polar, Hy-B | **3 (**Arg-741, Leu-147**)** |

H-B Hydrogen Bond, Hy-B Hydrophobic Bond, - ve Negative +ve Positive

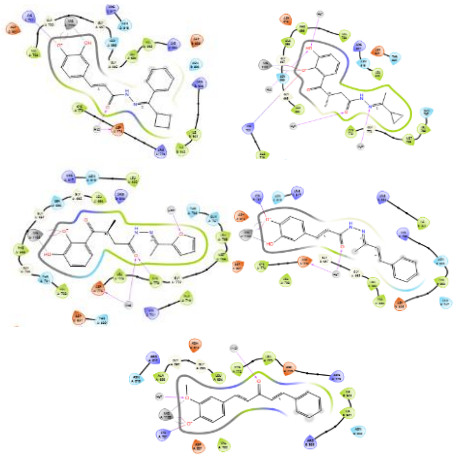

**Fig: 6 Interaction of selected ligands with EGFr**

**Table2: Free binding energy of docked complex**

| CC | $\Delta G$ binding | $\Delta G$ H bond | $\Delta G$ lipo | $\Delta G$ vdv | $\Delta G$ coulomb |
|---|---|---|---|---|---|
| **C68** | -61.68 | -4.54 | -24.25 | -32.20 | -25.35 |
| **C32** | -58.25 | -5.31 | -24.43 | -28.543 | -21.34 |
| **C61** | -58.42 | -4.82 | -26.52 | -29.44 | 9.51 |
| **C69** | -58.04 | -4.08 | -23.15 | -25.14 | 19.57 |
| **C26** | -59.35 | -5.12 | -25.23 | -29.43 | -15.25 |
| **C74** | -59.50 | -5.22 | -29.04 | -34.08 | 26.07 |
| **A35** | -42.78 | -2.83 | -27.37 | -40.29 | -11.91 |
| **A36** | -51.89 | -4.11 | -27.31 | -36.58 | -33.07 |
| **A31** | -43.95 | -2.37 | -26.01 | -39.86 | -15.08 |
| **A33** | -45.73 | -2.95 | -19.26 | -34.51 | -36.03 |

Physiochemical properties are shown in table (3). Most of the compounds fitted into the Lipinski R05 with any violations. Criteria of rule was set for identify drug like, lead like and fragment-like property. 50% of compounds showed drug like, 30% lead like and 15% fragment like as shown in table (4).

**Table3: Lipinski's rule of five for identified compounds**

| CC | Molecular weight | HBD | HBA | QPlog Po/w | TPSA | RO5 |
|---|---|---|---|---|---|---|
| **C68** | 304.345 | 2 | 4.5 | 2.767 | 121.16 | 0 |
| **C32** | 336.39 | 3 | 3.5 | 3.795 | 93.077 | 0 |
| **C61** | 330.34 | 2 | 5 | 2.869 | 123.112 | 0 |
| **C69** | 320.388 | 2 | 4..5 | 3.373 | 117.872 | 0 |
| **C26** | 322.363 | 3 | 3.5 | -5.215 | 92.775 | 0 |
| **C74** | 424.496 | 3 | 5.2 | 4.419 | 132.812 | 0 |
| **C35** | 359.384 | 3 | 4.5 | 3.705 | 104.51 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **A36** | 280.323 | 1 | 3.5 | 3.747 | 57.37 | 0 |
| **A31** | 244.246 | 1 | 4 | 2.302 | 66.929 | 0 |
| **A33** | 296.365 | 1 | 3.5 | 4.245 | 57.025 | 0 |

**Table4: Identification of drug like, lead like and fragment-like property**

| Criteria | Drug like | Lead like | Fragment like |
|---|---|---|---|
| Molecular weight | < 500 | 150 ≤ MW | MW ≤ 250 |
| Log p | < 5 | ≤ 4 | ≤3 |
| HBA | ≤ 10 | ≤ 3 | <3 |
| HBD | ≤ 5 | ≤ 6 | <6 |
| Satisfying % | 50% | 30% | 15% |
| % of Drugs | 45% | 39% | 16% |

**Table5: Predicted ADME parameters of in silico compounds**

| CC | SASA | FOSA | FISA | PISA | VOLUME | QPPCaco | QplogBB | HOA (1%) |
|---|---|---|---|---|---|---|---|---|
| **C68** | 622.48 | 316.18 | 182.06 | 124.231 | 1061.35 | 185.93 | -1.83 | 3 |
| **C32** | 665.89 | 179.89 | 166.64 | 319.359 | 1146.50 | 260.39 | -1.73 | 3 |
| **C61** | 626.38 | 175.14 | 180.17 | 271.07 | 1078.7 | 193.78 | -1.8 | 3 |
| **C69** | 649.57 | 371.66 | 158.3 | 119.557 | 1132.83 | 312.08 | -1.65 | 3 |
| **C26** | 673.50 | 116.55 | 174.03 | 382.918 | 1129.25 | 221.58 | -1.95 | 3 |
| **C74** | 725.50 | 269.46 | 186.20 | 269.832 | 1349.56 | 169.86 | -1.97 | 3 |
| **C35** | 690.22 | 16.61 | 185.23 | 488.377 | 1182.14 | 173.50 | -1.99 | 3 |
| **A36** | 586.03 | 130.62 | 97.6 | 357.721 | 985.96 | 1173.55 | -0.89 | 3 |
| **A31** | 491.97 | 105.72 | 107.33 | 278.904 | 814.09 | 950.62 | -0.77 | 3 |
| **A33** | 296.36 | 1 | 3.5 | 4.245 | 57.02 | 0 | 296.36 | 1 |

**Table6: Evaluation of Cardiotoxicity, Metabolism and skin permeability**

| CC | Herg K+ | Log Kp | Metabolic | Jm |
|---|---|---|---|---|
| **C68** | -5.22 | -3.573 | 4 | 0.003 |
| **C32** | -6.36 | -2.602 | 3 | 0.005 |
| **C61** | -5.86 | -3.021 | 6 | 0.015 |
| **C69** | -3.95 | -3.581 | 5 | 0.025 |
| **C26** | -6.94 | -2.418 | 3 | 0.008 |
| **C74** | -5.82 | -2.945 | 5 | 0.002 |
| **C35** | 0.006 | 0.006 | 0.006 | 0.006 |
| **A36** | -6.794 | -1.906 | 3 | 0.031 |
| **A31** | -5.931 | -2.355 | 4 | 0.172 |
| **A33** | -6.656 | -1.906 | 5 | 0.007 |

Oral availability was reported based on Jorgenson Rule of 3, log swat, CaCCo permeability test and no. of rotatable bonds suggest some degree of flexibility. Human oral absorption prediction was covered by 60% of compounds high but all were found within limits. 99% of compounds were found to be active in CNS fell within recommended range for predicted BBB partition coefficient and 65% of compounds displayed MDCK cell permeability. Skin permeability (log Kp) fell within predicted range log KHSA (-1.5 to + 1.5) showed variation > 96% compounds indicating free circulation within blood steam and target. Recommend no. of metabolic reaction was predicted and satisfied for 100% compounds and > then 75% satisfied the recommended range of IC50 for HERG K+ channel (> -5) (Table 5-6). The overall ADMET properties of compounds indicate a good pharmacokinetic profile.

## *Conclusion:*

Molecular docking and pharmacokinetic studies were carried out on 480 set of pyrimidines, Pyridine, pyrazoline and caffeic acid analogues as epidermal growth factor receptor tyrosine kinase inhibitors. This study confirmed the epidermal growth factor receptor tyrosine kinase inhibitory activities of the reported molecules, their safety through their pharmacokinetic profiles and could be further programmed for synthesis and invitro evaluation used as potential drugs for the treatment of EGFR in glioblastoma mutations.

### *Conflicts of interest*

The authors declare that there is no conflict of interest regarding the publication of this manuscript. All research and findings presented in this study are based solely on scientific data, and no financial or personal relationships have influenced the results or conclusions drawn.

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