MOLECULAR DOCKING INVESTIGATION AND PHARMACOKINETIC PROPERTIES PREDICTION OF SOME ANILINOPYRIMIDINES ANALOGUES AS EGFR T790M TYROSINE KINASE INHIBITORS

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ABSTRACT
The most common and deadly type of lung cancers around the globe was the non-small-cell lung cancer with approximately 1.5 million cases and a 5-year survival rate of less than 20%. Therefore, there is need for more active drugs to treat this problem. Molecular docking study was carried out on thirty two compounds of anilinopyrimidines analogues as epidermal growth factor receptor tyrosine kinase inhibitors using Molegro Virtual Docker (MVD). Ten (10) compounds with the best plant scores (binding affinities) among the compounds under investigation were reported in this study. All the ten compounds were observed to have plant score between −75.2644 to −94.2497, respectively. Their interactions in the active site of their target receptor (3IKA) were via hydrogen, hydrophobic, and electrostatic bonds sharing the following common amino acid residues LEU718, LEU844, PHE732, ALA743, VAL726, and MET793, respectively. This study also predicts the ADME properties of these compounds under investigation and found to have good pharmacokinetic properties. It further predicts their drug-likeness properties and found to be orally bioavailable with good bioavailability scores. It concluded that these compounds could be used as potential drugs for the treatment of EGFR{T790M/L858R} double mutations.

Introduction
Lung cancer remains the most common and deadly type of all cancers around the globe [1]. Lung cancer was estimated to account for about 25% of the 7 million people who died as a result of cancer-related issues/mortality every year in the world [2]. Lung was classified traditionally into small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) [3]. NSCLC was the most deadly class of lung cancer with approximately 1.5 million cases and a 5-year survival rate of less than 20% [4].

Receptor tyrosine kinases (RTKs) belong to the ErbB family, located on cell membranes of living organisms and played a significant role in the management of the physiological cycle of malignant tumors [5]. Epidermal growth factor receptor (EGFR) is a member of RTKs, which was recognized to be one of the most significant targets for the management of malignant tumors (such as lung cancer), which plays a vital role in the control of cancer cell growth, proliferation and differentiation [6,7]. It has an extracellular ligand binding domain, a transmembrane
portion, and intracellular tyrosine kinase and regulatory domains. Upon binding of a specific ligand (e.g., epidermal growth factor), the normal function of EGFR undergoes conformational change and phosphorylation of the intracellular domain occurs, leading to downstream signal transduction by various pathways. These include the Raf1-extracellular signal-regulated kinase, PI3K/Akt, and signal transducer and activator of transcription (STAT) factors. Depending on the pathway, the end result is cell proliferation or cell maintenance by inhibition of apoptosis [8].

DNA mutations in EGFR as detected by polymerase chain reaction (PCR) can occur in regions corresponding to the extracellular or intracellular portions of the protein [8]. The major mutations reported in EGFR TK occur in exon 19 deletion mutations and the single-point substitution mutation L858R in exon 21, which were the most frequent in NSCLC and were termed ‘classical’ mutations. It also occurs in exon 20 as a result of acquired resistance by the T790M mutation. Other mutations reported were D761Y, L747S, and T854A, respectively [9]. In non-small cell lung cancer, overexpressions of EGFR or mutations in intracellular EGFR have been observed in 43–89% of cases [8,10]. Other literature reported that one-quarter of NSCLC had mutations in the EGFR tyrosine kinase domain and these were associated with increased receptor expression in 75% of cases (35, 36). Of the known EGFR tyrosine kinase domain mutations, greater than 90% occur as short in-frame deletions in exon 19 or as point mutations in exon 21, the latter resulting in arginine replacing leucine at codon 858 (L858R) [8,11]. These mutations can result in constitutive activation of signal transduction pathways, leading to cell proliferation or anti-apoptosis, regardless of the presence of extracellular ligand. Two less common mutations occur at exons 18 and 21. Of note, EGFR and KRAS mutations appear to be mutually exclusive [8].

Many EGFR tyrosine kinase inhibitors share a common pharmacophore and structure of quinazoline and acrylamide. A lot of EGFR tyrosine kinase inhibitors have been designed and developed starting from first, second and up to third generation. The first-generation EGFR tyrosine kinase inhibitors were developed to treat patients with EGFR mutation caused by the L858R mutation [12]. The first-generation EGFR tyrosine kinase inhibitors were reported to have a remarkable therapeutic effect in the early stage of clinical treatment, in the first year of treatment with these drugs more than 50% of patient developed resistance to these drugs in more than half of the cases caused by a secondary mutation T790M, the so-called ‘gatekeeper’ mutation [13,14].

As such, many second-generation EGFR tyrosine kinase inhibitors were developed to manage the resistance caused by the T790M mutation such as afatinib and docatinib. However, these inhibitors cannot achieve advantages over the first-generation reversible inhibitors due to the serious side effects, such as skin rash, diarrhea. It is thought that the activities against wild-type (WT) EGFR will limit the achievable activities against the T790M mutation in patients [15].

Many third-generation EGFR inhibitors such as AZD9291, CO1686, and WZ4002 were developed to inhibit T790M resistance mutation while being more selective for wild type EGFR (WT EGFR). In preclinical studies, these inhibitors were tested in both enzyme activities assays for L858R/T790M and WT EGFR, and cellular inhibition assays for NCI-H1975 cells harboring EGFR L858R/T790M mutations and A431 cells expressing WT EGFR [16].

The aim of this work is to carry out molecular docking investigation on some anilinopyrimidines analogues as EGFR T790M/ L858R double mutant inhibitors and predict their pharmacokinetic properties.

Materials and methods

Compounds selection and drawing of 2D-structures

Thirty two (32) anilinopyrimidines analogues were synthesized and reported by Romu et al.
Out of these 32 compounds, 20 compounds were evaluated for their percentage inhibition at 1µ and 10µ against six different EGFR kinases (EGFRWT, EGFRd746–750, EGFRT790, EGFRT790M/L858R, EGFRC797S and EGFRT790M/C797S/L858R). Then, all the 32 compounds were then evaluated to determine their cytotoxic activities against three different human non-small lung cancer cells (PC9, PC9GR and H460) and mouse leukemic cells (Ba/F3 WT and Ba/F3 T3151). In their cytotoxicity assay, majority of the target compounds have similar or lower IC50 values against PC9-GR, H460, Ba/F3 WT and Ba/F3-T3151 cell lines in comparison with the lead compound WZ4002. Chemdraw software developed by Cambridge University was used to draw the 2D-structures of the selected compounds [18,19]. The structural formulas of the reported compounds are presented in supplementary Table 1.

Conversion of 2D to 3D structures and stable conformation search

The conversion of 2D to 3D structures of the anilinopyrimidines analogues was carried out by direct importation of the structures on to the Spartan 14 software interface. The search for the stable conformation of all the anilinopyrimidines analogues was performed using Merck molecular force field (MMFF) with density functional theory (DFT) at Becke’s three-parameter hybrid function utilizing LYP correlation functional using 6–311 G* basis set [20]. Then, the stable conformations of the anilinopyrimidines analogues were saved in protein data bank file format [21,22]. Figure 1 presents the 3D structure of stable conformation of an anilinopyrimidine analogue.

Active site identification

The crystal structure of EGFR enzyme with pdb entry codes 3IKA covalently binding to WZ4002 was downloaded from the RCSB protein data bank database (https://www.rcsb.org/) [23]. In order to identify the amino acid residues in the active site of the enzymes, the enzymes were visualized with discovery studio since they came with co-crystalline ligands. The amino acid residues identified in the active site of 3IKA were MET793, ALA743, MET790, LEU844, LEU718, and VAL726, respectively [24]. Figure 2 shows the co-crystallized ligand lying in the active site of 3IKA receptor.

Molecular docking execution

Molegro Virtual Docker (MVD) was used to carry out protein-ligand docking investigation in this research due to its high accuracy compared to other docking programs. Prior to the molecular docking execution, the enzyme was imported on the MVD and then residues with structural error were repaired/rebuilt. In the course of preparing the enzyme, surface was created and cavities were detected before removing the co-crystalized ligand from the enzyme. The ligands were all prepared by the default setting by; if missing to; assign bonds, assign bond orders and hybridization, assign charges and assign tripos atom types as well as creating explicit hydrogens and detecting flexible torsions in the ligands. Then, execution of the docking process was done by selecting plant score as the docking algorithm (scoring function) and defining the binding site as a spherical region which consists of all the protein cavities within 22 Å of the ligand atom with X, Y, and Z coordinate for this current work. For all other calculations, default setting was maintained. The Docking was performed using a grid resolution of 0.30 Å and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The docking process was validated by docking re-docking WZ4002 into the active sites of 3IKA receptor. The residues of the docked co-crystallized ligands were then compared to those of the compounds under investigation [25].
Table 1. The plant scores and different types of interaction of some selected compounds in the active site of the 3IKA receptor.

| S/No | Plants Score | Hydrogen bond interactions | Hydrophobic and electrostatic interactions |
|------|--------------|----------------------------|--------------------------------------------|
|      |              | Types of Amino acids interaction | Types of Amino acids Interaction |
| 1    | −85.913      | ALA743, LEU792, MET793 & Alkyl LEU844 | PHE723, VAL726, LYS745, Pi-Alkyl VAL726, ALA743, LEU844, LEU718 & CYS797 |
| 3    | −77.8063     | MET793 & MET793 Conventional H. Bond | ASP855 Pi-Anion |
|      |              |                             | PHE723 Pi-Pi Stacked |
|      |              |                             | ALA743, LEU792, MET793 Alkyl & LEU844 |
|      |              |                             | PHE723, VAL726, VAL726, Pi-Alkyl ALA743, LEU844 & LEU718 |
| 9    | −78.65       | LYS745, ARG841, Conventional H. Bond ARG841 & ASP855 | ASP855 Pi-Anion |
|      |              | GLN791 & GLN791 Carbon H. bond | PHE723, VAL726, Alkyl LEU718 & LYS745 Pi-Alkyl |
| 10   | −90.0224     | ASP800 Carbon H. bond | ASP855 Pi-Anion |
|      |              |                             | PHE723 Pi-Pi Stacked |
|      |              |                             | LEU718 & GLY719 Amide-Pi Stacked |
|      |              |                             | PHE723, VAL726, VAL726, Pi-Alkyl LEU718 & LEU844 |
| 15   | −80.8012     | ASP800 Carbon H. bond | GLY796 Pi-sigma |
|      |              |                             | MET790 & MET793 Pi-Sulfur |
|      |              |                             | CYS797, LEU799, ARG841, Alkyl LEU718 & LEU792 |
|      |              |                             | CYS797, LEU718, LEU844, Pi-Alkyl ALA743 & LEU844 |
| 17   | −84.7912     | LYS745 & LYS745 Conventional H. Bond PRO794, PRO794 Carbon H. Bond & PHE723 | MET790 Pi-Sulfur |
|      |              |                             | LEU718, LEU792 & MET790 Alkyl LEU718, LEU844 & LEU844 Pi-Alkyl |
| 18   | −94.2497     | PHE795 Carbon H. bond | TYR801 Pi-Pi Stacked |
|      |              |                             | ALA743, LEU718, LEU792 Alkyl & MET793 |
|      |              |                             | PHE723, VAL726, LEU718, Pi-Alkyl VAL726, ALA743, LEU844, LEU718 & CYS797 |
| 20   | −86.8363     | PRO794 & GLN791 Carbon H. Bond | ASP855 Pi-Anion |
|      |              |                             | LEU718 Pi-Sigma |
|      |              |                             | MET790 & CYS797 Pi-Sulfur LEU718, CYS775, MET790 Alkyl & LEU844 |
|      |              |                             | ALA743, MET793, LEU844, Pi-Alkyl LEU799 & ARG841 |
| 29   | −88.0704     | LYS745 & ARG841 Conventional H. Bond ILE878 Carbon H. Bond | ASP855 Pi-Anion |
|      |              |                             | PHE723 Pi-Pi Stacked |
|      |              |                             | VAL726, ALA743 & LEU844 Alkyl VAL726, PRO877, LYS879 & Pi-Alkyl ALA920 |
| 30   | −79.8455     | MET793 Conventional H. Bond GLU762, ASP855 Carbon H. Bond & MET793 | ASP855 Pi-Anion |
|      |              |                             | CYS797 Pi-Sulfur |
|      |              |                             | CYS797, LEU799 & ARG841 Alkyl PHE723, ARG841 & CYS797 Pi-Alkyl |
| WZ4002 | −79.9418   | MET793 Conventional H. Bond | PHE723 Pi-Pi Stacked |
|      |              |                             | LEU718 Alkyl PHE723, VAL726, LEU718 Pi-Alkyl VAL726, LEU718 & LEU844 |
Pharmacokinetics studies

The pharmacokinetics/ADMET properties of these compounds under investigation were determined using pkCSM an online web server (http://structure.bioc.cam.ac.uk/pkcsms), which uses graph-based signatures to generate predictive models of central ADMET properties for drug discovery. While the drug-likeness of these compounds were also evaluated using SWISSADME (http://www.swissadme.ch/index.php) an online web tool. Many rules were developed to guide the choice of compounds in the early phases of drug discovery. Among the famous rules applied for selection of compounds based on the drug-likeness properties in early phase of drug discovery were the Lipinski’s rule of five (RO5). The studied compounds would be evaluated for their drug-likeness properties based on the RO5 criteria [26,27]

Results and discussion

Molecular docking

Molecular docking investigation was carried out between thirty two (32) series of anilinopyrimidines analogues and the active site of 3IKA receptor using MVD due to its high accuracy in determining ligand-receptor interaction as compared to other docking programs. All the 32 compounds of anilinopyrimidines analogues showed good docking affinities (plant scores) ranging from −75.2644 to −94.2497, respectively. The plants scores, hydrogen bond, hydrophobic and electrostatic interactions of all the anilinopyrimidines analogues were shown in the Supplementary Table 2. The result of 10 selected anilinopyrimidines analogues will be discussed (Table 1). To validate the docking protocol in this study, WZ4002 was also redocked into the active site of 3IKA receptor and then compared with those of the reported

Figure 1. The 3D structure of stable conformation of compound 1 using discovery studio.

Figure 2. The co-crystalized ligand in the active site of 3IKA receptor.
compounds of anilinopyrimidines analogues (Table 1). Among the reported compounds, compound 18 has the best plant score of −94.2497. The compound was observed to involve carbon hydrogen bond with PHE795 at a distance of 2.61 Å in the active site of 3IKA receptor. It was also observed to involve Pi-Pi Stacked hydrophobic interaction with TYR801, Alkyl hydrophobic interaction with ALA743, LEU718, LEU792, MET793, and Pi-alkyl hydrophobic interaction with PHE723, VAL726, LEU718, VAL726, ALA743, LEU844, LEU718, and CYS797 amino acid residues in the active site of the receptor, respectively. The 3D and 2D structures of compound interacting in the active site of 3IKA receptor are shown in Figure 3.

The second best among the compounds under investigation was compound 10 with a plant score of −90.0224. The compound was found to involved carbon hydrogen bonding with ASP800 with bond distance of 3.08 Å. Beside the carbon hydrogen bonding, it was seen to involve interaction with ASP855 amino acid through Pi-Anion hydrophobic interaction, with PHE723 amino acid through Pi-Pi stacked hydrophobic interaction, with LEU718 and GLY719 amino acids through Amide-Pi stacked hydrophobic interaction and via Pi-Alkyl hydrophobic interaction with PHE723, VAL726, VAL726, LEU718, and LEU844 amino acid residues,

Table 2. The predicted ADMET properties of the reported compounds.

| Substrate | Intestinal absorption (human) | BBB permeability (Log BB) | CNS perm. (Log PS) | Substrate Inhibitors | CYP | Total Clearance | TOXICITY | AMES toxicity |
|-----------|-------------------------------|--------------------------|-------------------|---------------------|-----|----------------|---------|---------------|
| 1         | 84.208                        | −1.286                   | −2.605            | No                  | Yes | No            | Yes     | No            |
| 3         | 88.68                         | −1.082                   | −2.436            | No                  | Yes | No            | No      | No            |
| 9         | 74.66                         | −1.391                   | −3.62             | No                  | Yes | No            | No      | No            |
| 10        | 97.459                        | −1.489                   | −3.15             | No                  | Yes | No            | Yes     | Yes           |
| 15        | 87.611                        | −1.107                   | −2.235            | No                  | Yes | No            | No      | No            |
| 17        | 83.531                        | −1.244                   | −2.763            | No                  | Yes | No            | Yes     | No            |
| 18        | 93.66                         | −1.44                    | −3.219            | No                  | Yes | No            | Yes     | No            |
| 20        | 94.419                        | −1.484                   | −3.175            | No                  | Yes | Yes           | Yes     | No            |
| 29        | 90.687                        | −1.443                   | −3.229            | No                  | Yes | No            | Yes     | No            |
| 30        | 76.641                        | −1.404                   | −3.548            | No                  | Yes | No            | Yes     | No            |

Figure 3. 3D and 2D visualizations of compound 18 in the active site of 3IKA receptor with discovery studio.
respectively. Figure 4 presents the compound in the active site of the receptor in 3D and 2D form.

Another compound with a good plant score of −88.0704 among the reported compounds was compound 29. It involves conventional hydrogen bond interaction with LYS745 and ARG841 amino acid residues in the binding pocket of the 3IKA target with bond distances of 1.97 Å and 2.56 Å each. It also involved Pi-Pi Stacked, Alkyl and Pi-Alkyl hydrophobic interactions with the following amino acid residues PHE72, VAL726, ALA743, LEU844, VAL726, PRO877, LYS879, and ALA920, respectively. The compound further interacted in the binding pose of the target via Pi-Anion electrostatic with ASP855 amino acid. Figure 5 shows the 3D and 2D visualization of the ligand in the active site of the receptor.

Compound 20 also showed good affinity toward the target receptor with a plant score of −86.8363 and was observed to form carbon hydrogen bond with PRO794 and GLN791 amino acid residues each with distance of 2.46 Å and 2.28 Å, respectively. Pi-Anion electrostatic interaction was also observed between the compound and ASP855, MET790, and CYS797 amino acids in the binding pose of the target receptor. Apart from the two mentioned interactions, Pi-sigma, Pi-Sulfur, Alkyl, and Pi-Alkyl hydrophobic interactions were also observed with LEU718, LEU718, CYS775, MET790, LEU844, ALA743, MET793, LEU844, LEU718, LEU718, CYS775, MET790, LEU844, ALA743, MET793, LEU844,
LEU799, and ARG841 amino acid residues, respectively. Figure 6 shows the compound in active site of the receptor with interacting residues.

Compound 1 with a plant score of −85.913 was also among the reported compounds with good affinities, which formed Alkyl and Pi-Alkyl hydrophobic interactions in the active site of the target receptor with ALA743, LEU792, MET793, LEU844, PHE723, VAL726, LYS745, VAL726, ALA743, LEU844, LEU718, and CYS797 amino acid residues as shown in Figure 7, respectively.

To validate the accuracy of the docking protocol, the co-crystalized ligand (WZ4002) was also re-docked to the active site of the 3IKA target receptor to see whether the reported compounds fit well in the active site of the target. The co-crystalized ligand with plant score of −79.7418 was found to interact with the following amino acid residues in the active site of the target: MET793, PHE723, LEU718, PHE723, VAL726, LEU718, VAL726, LEU718, and LEU844, respectively (Figure 8). The epidermal growth factor receptor is a well-validated target for lung cancer therapy, many researchers in different literatures had used EGFR as target in lung cancer therapy, to mention but few the work reported by [23, 28–30], respectively.

The following LEU718, LEU844, PHE732, ALA743, VAL726, and MET793 amino acid residues were virtually common to all of the
reported compounds including the WZ4002. All other reported compounds were also seen to have good plant scores as shown in Table 1. The compounds were observed to interact with the active site of the target receptor involving conventional, carbon hydrogen bond, electrostatic, and hydrophobic interactions, respectively.

**Pharmacokinetic studies**

The pharmacokinetic parameters ADMET and drug-likeness of all the anilinopyrimidines analogues were predicted to confirm the viability of the drugs (Supplementary Tables 3 and 4) employing pkCSM and SWISSADME online web tools. The ADMET properties of the reported compounds are shown in Table 2 and the drug-likeness properties of the reported compounds are presented in Table 3.

All the reported compounds have absorbance value between 76.641 and 94.419% as the values passed the minimum recommended values of 30% which indicates good human intestinal absorption. The minimum recommended values for the blood–brain barrier (BBB) and central nervous

**Table 3.** The predicted drug-likeness properties of the reported compounds.

| Molecule | MW  | No. of H-bond acceptors | No. of H-bond donors | MLOGP | No. of Lipinski’s violations | Bioavailability Score | Drug-likeness |
|----------|-----|-------------------------|----------------------|-------|----------------------------|----------------------|---------------|
| Molecule 1 | 494.97 | 6                       | 2                    | 2.4   | 0                          | 0.55                 | YES           |
| Molecule 3 | 440.93 | 5                       | 2                    | 2.29  | 0                          | 0.55                 | YES           |
| Molecule 9 | 498.96 | 7                       | 3                    | 1.49  | 0                          | 0.55                 | YES           |
| Molecule 10 | 603.11 | 7                       | 2                    | 2.84  | 1                          | 0.55                 | YES           |
| Molecule 15 | 482.99 | 6                       | 1                    | 3.94  | 0                          | 0.55                 | YES           |
| Molecule 17 | 552.07 | 7                       | 2                    | 2.22  | 1                          | 0.55                 | YES           |
| Molecule 18 | 589.08 | 7                       | 2                    | 2.65  | 1                          | 0.55                 | YES           |
| Molecule 20 | 603.11 | 7                       | 2                    | 2.84  | 1                          | 0.55                 | YES           |
| Molecule 29 | 589.08 | 7                       | 2                    | 2.65  | 1                          | 0.55                 | YES           |
| Molecule 30 | 498.96 | 7                       | 3                    | 1.49  | 0                          | 0.55                 | YES           |
system permeability is $> 0.3$ to $<-1$ Log BB and $>-2$ to $<-3$ Log PS, respectively. As for these compounds, Log BB is $>-1$ for all which implies that the compounds are better distributed to brain and Log PS for all is $>-2$, which are considered to penetrate the central nervous system. The enzymatic metabolism of drugs shows the biotransformation of a drug in the body. It is, therefore, very important to put into consideration the metabolism of drugs, as such the cytochrome P450 plays an important role in drug metabolism. CYP families involved in drug metabolism were 1A2, 2C9, 2C19, 2D6, and 3A4, respectively. The most important among the mention CYP families is 3A4 which all the reported compounds were found to be substrate and inhibitors of it. Total clearance is an indicator, which describes the relationship between the rate of elimination of the drug and its concentration in the body. The reported compounds showed high value of total clearance but within the accepted limit of a drug molecule in the body. Furthermore, all the reported compounds were found to be nontoxic. The overall ADME properties of these compounds indicate their good pharmacokinetic profiles (Table 2).

Compounds 1, 3, 9, 15, and 30 among the reported compounds respect the Lipinski’s rule of five without violating any of the condition set by the rule (Molecular weight $\leq$ 500, Number of hydrogen bond donors $\leq$ 5, Number of hydrogen bond acceptors $\leq$ 10, and Calculated Log p $\leq$ 5). While other reported compounds were found to have one violation (that is their molecular weight was greater than 500) but still within the permissible limit for drug molecule to be orally bioavailable. All the compounds have good bioavailability score of 0.55 which further confirmed the drug-likeness properties of these reported compounds (Table 3). As such the reported compounds are said to be orally bioavailable with good pharmacokinetic profiles.

**Conclusion**

Molecular docking and pharmacokinetic studies were carried out on 32 set of anilinopyrimidines 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 used as potential drugs for the treatment of EGFR in non-small-cell lung cancers (NSCLC) mutations.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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