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MOLECULAR DOCKING AND *IN-SILICO* ADME STUDIES OF NOVEL DERIVATIVE OF ERLOTINIB IN GLIOMA

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ABSTRACT: Glioblastoma (GBM), with restricted therapy alternatives, is a catastrophic primary brain tumor. The receptor of the epidermal growth factor receptor (EGFR) in glioblastomas is recurrently enhanced, over articulated, or mutated, but up to 20 percent of GBM patients find it to be responded to kinase inhibition of EGFR. Several inhibitors of EGFR tyrosine kinase (TKI) failed clinically, due in part to acquired resistance. To automatically examine this type of resistance, we used molecular docking and swissADME approach to elucidate its putative inhibitor. We have attempted to determine a drug candidate in the current research based on the discovery of structural drugs. Docking simulation was conducted on mutated EFGR to determine the best drug candidate from Erlotinib, a renowned anti-cancer agent, derivatives. A total of 200 structures were selected for the 2D crystal structure of erlotinib based on molecular fingerprinting. Top 10 best-docked proteins were analyzed using UCSF Chimera and discovered the complicated atomic-scale properties between ligand and the target protein. SCHEMBL13087058 ligand selected based on hydrogen bonding with methionine and swissADME screening shown the drug likeliness of the molecule with Molecular docking results showed binding energy -14.29 kcal/mol. Further wet lab study requires to study the actual binding as compulsory mode provided. To discover new inhibitors of EGFR with higher potency and specificity, additional information is needed for future design molecules.

INTRODUCTION: Nonetheless, the advanced cancer therapy Glioblastoma multiforme (GBM), known to be highly invasive and aggressive among cancer of the CNS (Central nervous system). Glioblastoma arises from progenitor cells of astrocytes and is found to be the most aggressive [1-3]. After tremendous cancer research, it is still showing challenges in early prognosis and treatment.

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GBM comprising around 80% malignancies among adults with its most miserable prognosis record [4, 5]. To date, in combination with postoperative radiotherapy and chemotherapy, GBM treatment is constructed on maximum surgical excision. GBM generally found to be highly chemo and radiotherapy resistive in nature, so the clinical intervention is confined and majorly restricted [6].

Although GBM diagnosis and therapy methods have been expressively enhanced, the phase IV survival of patients with glioblastoma is only 1-2 years yet, with a survival rate of less than 6% for five years [7]. There is a desperate need to look out new treatment opportunities for a better cure. EGF receptor is recommended as a striking hallmark of glioblastoma, so it is established as an anti-GBM

therapy target. However, GBM has feeble responses to the first-generation EGFR inhibitors. A recent development in GBM signaling revealed the role of RTK/PI3K/R as a pathway in cancer, any alteration in these pathways could lead to high expression of the EGFR pathway and functional loss of PTEN (Phosphatase and Tensin homolog) [8-10]. PTEN is widely known as a tumor suppressor and controls cell division. EFGR and PTEN found to be regulating the Akt/PI3K pathway [11].

It is found that EGF receptor inhibitors like erlotinib decrease *in-vitro* proliferation of cells, motility, and cell invasion [12, 13]. Tumor cell proliferation, differentiation, migration, and homeostasis are regulated by atypical expression of EGFR [14]. About 57% of GBMs have genetic alternates of EGFR, those include including mutations, rearrangements, splicing, and amplification [15, 16]. In divergence to lung carcinoma, with EGFR mutations mainly in the kinase region, GBM shows EGFR mutations primarily in the extracellular areas [17]. The EGFR mutations allow GBM cells to trigger downstream signaling pathways PI3 K/AKT and RAS/ERK independent of EGF ligands. Numerous studies have shown that EGFR overexpression and mutation support GBM development and survival [18, 19]. The EGF receptor gene knockout revealed that the survival of EGFR-mutant glioblastoma cells depends on the function of EGF receptor [17]. Therefore, EGFR has long been regarded as a very appealing target for glioblastoma therapy. Erlotinib had an efficient sensitivity in pre-clinical studies by suppressing anchorage-independent growth in tumor cell lines [20]. Therefore, we will concentrate on this article on the EGFR-targeted methods to solve the therapeutic resistance issue.

MATERIALS AND METHODS:

Ligand Selection: 2D structural equivalent N-(3-Ethynylphenyl)-6, 7-bis (2-methoxy ethoxy) quinazoline-4-inbuilt PubChem database search for similarity fingerprints. There were chosen a total of 500 ligands with the lowest score of 0.68 in which only 200 were lastly tested for docking. ACD / ChemSketch instrument was used for MDL Molfile (v2000) generation and drawing. Molefile converted into a 3D database of proteins (PDB) format using the Bebel tool. Erlotinib was obtained based on the resemblance between its composition, substructure, and chemical isomers.

Receptor Protein: The 3D PDB crystal assembly of the EGFR tyrosine kinase domain with novel HER2 active site inhibitor, downloaded from RCBS (http:/www.rcsb.org) using PDB ID: 5JEB. Dedicated ligands were divided and energy minimization introduced through the optimized Swiss PDB viewer protocol [21].

Docking Setup: Protein and ligand docking experiments were conducted using Auto Dock software version 4, which demonstrates the binding energy analysis through grid and energy potential using different search algorithms to determine precise binding features on the specified super molecule [22]. Using the AutoDock hydrogen module, polar hydrogen molecules are added to the ligand. A conventional method was used for the distribution of the Kollman united partial atom charges involving random areas with a population size of 150.

The result was that 1 root-mean-square deviation standard with precise ten docking runs was rendered clustered. The grid size-12.352* 104.011* was chosen with 22.946 points and grid positioning of 0.375. The Chimera UCSF was used to view the docked structure coordinates within the range of 5 of the interaction between molecule and protein. The following URL (www.cgl.ucsf.edu/chimera) shows UCSF Chimera. Using Lipinskin "Rule of Five" to detect possible pharmacokinetics, the molecules that show the least binding energies were estimated for dug-likeness. All molecules undergo the analysis of molecular characteristics and scores of drug likeliness.

***In-silico* Bioavailability Analysis:** All ten ligands are checked using the swissADME tools available on the Swiss Institute of Bioinformatics page (http:/www.swissadme.ch/) for virtual physico-chemical features like water-solubility, lipophilicity, pharmacokinetics, drug resemblance and medicinal chemistry parameters [23]. All ten ligands were used, some of them showing the significant bioavailability.

RESULTS AND DISCUSSION:

Molecular Docking: Lamarckian Genetic Algorithm has been used to dock various erlotinib derivatives. The combination of a distinctive algorithm rule for binding site identification and

picture tool evaluation has given an effective way to exploit unrevealing binding cavity characteristics. The origin of the structural similarity of erlotinib was efficiently tested for a total of 200 molecules. The top 10 molecule data is shown below **Table 1**. Two SCHEMBL14405650 and SCHEMBL13087058 ligands showed the lowest minimum binding energy-17.56 and-14.29, respectively, with four and six runs.

Based on the Hydrogen bond formation with active site residue methionine of the receptor protein, the ligand SCHEMBL13087058 chosen as the best candidate among the top ten molecules. **Fig. 1**.

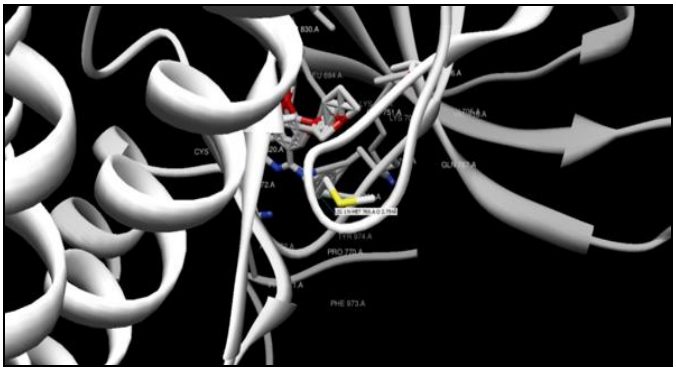
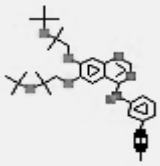
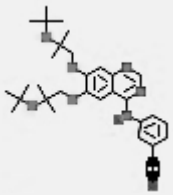
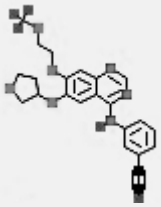
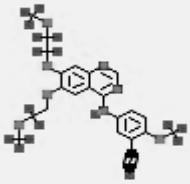
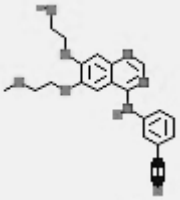
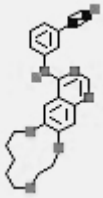
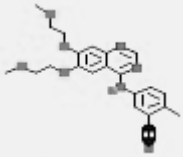
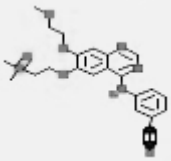
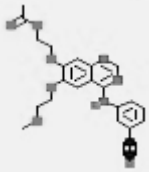
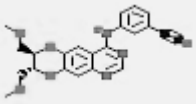


FIG. 1: THE BLUE LINE IN THE ABOVE PICTURES SHOWS HYDROGEN BOND WHICH INDICATES BONDING BETWEEN LIG 1 N-MET 769 WITH BOND LENGTH 2.754 Å

TABLE 1: CHEMICAL PROPERTIES OF THE TOP 10 MOLECULES WITH THE MINIMUM BINDING ENERGY

S. no.	Chemical Name	Structure	Minimum Binding energy	Run	PubChem CID
1	SCHEMBL14405650 6,7-Bis[2-methyl-2-[(2-methylpropan-2-yl)oxy]propoxy]-N-(3-prop-1-ynylphenyl)quinazolin-4-amine		17.56	4	71164834
2	SCHEMBL13087058 N-[3-(2-Deuterioethynyl)phenyl]-6,7-bis[2-methyl-2-[(2-methylpropan-2-yl)oxy]propoxy]quinazolin-4-amine		-14.29	6	59533121
3	N-(3-Ethynylphenyl)-6-(oxolan-3-yloxy)-7-[2-(trideuteriomethoxy)ethoxy]quinazolin-4-amine		-11.36	10	53311750
4	6-[2,2-Dideuterio-2-(trideuteriomethoxy)ethoxy]-N-[3-ethynyl-4-(trideuteriomethoxy)phenyl]-7-[1,1,2,2-tetradeuterio-2-(trideuteriomethoxy)ethoxy]quinazolin-4-amine		-11.92	4	44243270
5	UNII-EBL2O556JZ EBL2O556JZ 183320-29-8 Erlotinib metabolite M13 CP-373413		-13.33	9	16045730
6	N-(3-Ethynylphenyl)-2,5,11-trioxa-16,18-diazatricyclo[10.8.0.014,19]icosa-1(12),13,15,17,19-pentaen-15-amine		-11.42	6	10216002

7	4-Methyl Erlotinib Hydrochloride		-11.72	9	71750333
8	6-(2-Dimethylphosphorylethoxy)-N-(3-ethynylphenyl)-7-(2-methoxyethoxy)quinazolin-4-amine		-11.85	8	59068670
9	7-(2-Acetoxy-ethoxy)-4-(3-ethynyl-phenylamino)-6-(2-methoxy-ethoxy)-quinazoline		-14.65	9	16045729
10	(7R,8R)-N-(3-Ethynylphenyl)-7,8-bis(methoxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-g]quinazolin-4-amine		-11.58	9	11523983

ADME Test: Drug development includes increasingly previous evaluation stats of ADME (absorption, distribution, metabolism, and excretion) in the discovery procedure. Molecule SCHEMBL13087058 is evaluated for the Swiss ADME. Unique to swissADME is the bio-availability radar that provides a graphical snapshot of the drug-likeness parameters of an orally available bioactive drug. The drug-like graph is described as a hexagon **Fig. 1**, each of which represents a parameter defining a bioavailable drug. The pink area in the hexagon characterizes the optimum assortment for each property (lipophilicity: XLOGP3 6.51: MW 533.70 g / mol, polarity: TPSA 74.73 $Å^2$, solubility: log s 6, saturation value: 0.5 carbon portion in the sp3 hybridization and 12 rotatable bonds discovered flexibility) **Fig. 2**.

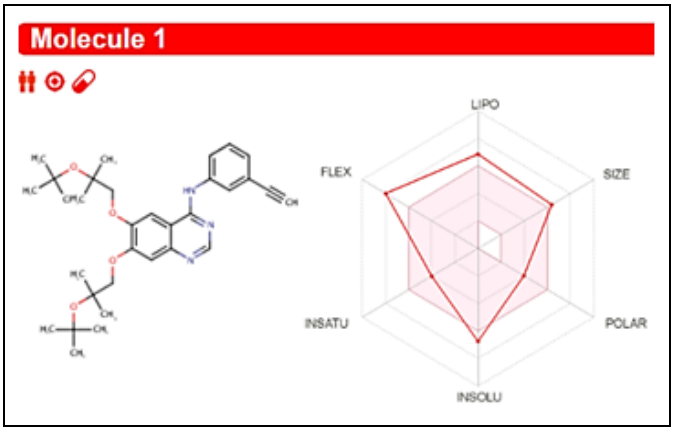


FIG. 2: SwissADME STRUCTURAL FEATURES AND BIOAVAILABILITY RADAR OF LIGAND SCHEMBL13087058

Drug-likeness: SCHEMBL13087058 drug-likeness properties are represented by the distorted red hexagon within the pink shade. Notably, the molecule falls within drug-likeness parameters of bioavailable drugs and following Lipski rule with bioavailability score of 0.55. SwissADME also has computational filters that include the Lipinski rule is the most common parameter on the way to evaluate the drug-likeness of small molecules **Fig. 3**.

Medicinal Chemistry Evaluation: Medicinal chemistry evaluation predicts the drug-likeness of small molecules, which include PAINS and Brenk screening rules. PAINS computer screening model identifies compounds that appear in many high-performance biochemical screens as hits (promiscuous compounds).

Druglikeness	
Lipinski	Yes; 1 violation: MW>500
Ghose	No; 4 violations: MW>480, WLOGP>5.6, MR>130, #atoms>70
Veber	No; 1 violation: Rotors>10
Egan	No; 1 violation: WLOGP>5.88
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: triple_bond
Leadlikeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	4.39

FIG. 3: SHOWING MEDICINAL DRUG LIKELINESS AND THERAPEUTIC CHEMISTRY EVALUATION

Our molecule does not post any pains alert. In another selection model, this molecule followed the Brenk screening law with only having three hydrogen bonds, which careful that compound is smaller and less hydrophobic and not those defined by "Lipinski's rule of 5". Due to the heavier molecular weight (MW>350), our ligand does not follow the lead likeness screening **Fig. 3**.

Pharmacokinetics: SwissADME also makes it possible to estimate a chemical as a p-glycoprotein (P-gp) substrate or CYP isoenzyme inhibitor. Drug metabolism through CYP isoenzymes is a significant determinant of drug contacts that may result in drug harmfulness and reduced pharmacological outcomes. The models reflect either yes or no if the molecule below review is more likely to be a substrate or non-substrate of a specified CYPP-gp substrate or an inhibitor or non-inhibitor. By inhibiting CYP2C19 inhibitor and CYP2D6, our ligand showed the pharmacological property. This molecule showed the low Gi absorption, and Log Kp (skin permeation) falls in a negative range of -4.93 cm/s. Ligand does not cross the blood-brain barrier. Now, a biological experiment will be required to determine if SCHEMBL13087058 is activated or deactivated by CYP2C9 and CYP3A4 **Fig. 4**.

	Pharmacokinetics
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.93 cm/s

FIG. 4: THE BIOAVAILABILITY RADAR OF THE SCHEMBL13087058 (LEFT PANEL) AND PHARMA-COKINETICS (RIGHT PANEL) EVALUATED USING SWISSADME WEB TOOL

CONCLUSION: Small molecules that block the altered metabolism in cancer are developing as latent anti-cancer agents. Erlotinib derivatives such as SCHEMBL13087058 can be used for anti-EGFR therapy against GBM. Indeed, it was more potent than clinically-tested in the Phase II trial. We used the molecular docking for shortlisting more than 200 molecules and found SCHEMBL13087058 most potent. Virtual tools swissADME evaluation revealed for the hit compound and demonstrated that SCHEMBL13087058 has better "drug-likeness".

Additionally, the ligand is lipophilic but does not penetrate the blood-brain barrier (BBB) and not a substrate of most CYP enzymes. Of note is the moderate synthetic accessibility of SCHEMBL-13087058 that provides opportunities for inhibiting GBM. Importantly, SCHEMBL13087058 did not show false-positive alert enabling us to rule out wrong targets with the confidence of pursuing potential biologically relevant targets. The real wet-lab experiment will help to take this molecule as a potent GBM target.

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CONFLICTS OF INTEREST: Authors declare no conflicts of interest.

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