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Beyond Binding Affinity: Integrated Docking and Pharmacokinetic Evaluation of EGFR-Targeting Natural Compounds in Glioblastoma

Abstract: The glioblastoma multiforme (GBM) is an aggressive primary brain tumour with a dismal prognosis, resistance to treatment, and high prevalence of deregulated Epidermal Growth Factor Receptor (EGFR) signalling. Clinically approved EGFR tyrosine kinase inhibitors (TKIs) like erlotinib have therapeutic potential, but their effectiveness in GBM is restricted by mechanisms of resistance and poor penetration of the central nervous system. For the present study, an integrated computational workflow was used which integrated molecular docking, residue-level interaction analysis and in silico pharmacokinetic analysis in order to assess selected natural compounds as potential EGFR targeting candidates. The crystal structure of the EGFR tyrosine kinase domain was downloaded from the RCSB Protein Data Bank (PDB ID: 1M17) and further prepared using AutoDock Tools. The molecular docking was carried out with AutoDock Vina with erlotinib as a reference inhibitor. The docked complexes were analysed for interaction using Discovery Studio Visualizer and PlexView, and the pharmacokinetic and drug-likeness properties were analysed using SwissADME. The most highly predicted binding affinity for EGFR of the screened compounds was diosquinone (−10.1 kcal/mol), which had a higher predicted binding affinity than erlotinib (−7.2 kcal/mol). Residue-level interaction analysis, however, indicated that diosquinone does not have the classical binding interactions for the hinge-region of the ATP binding site characteristic of erlotinib. Rather, alternative non-covalent interactions, such as cation–π interactions, seemed to be responsible for ligand stabilisation. Pharmacokinetic assessment showed good drug-likeness properties and low predicted penetration into the CNS, suggesting low exposure to the CNS even though there is high affinity for the receptor. In summary, the results show that docking affinity is not necessarily a good indicator of biologically relevant EGFR inhibition and underscore the need to incorporate docking geometry and pharmacokinetic analysis into computational drug discovery pipelines. The scaffold of diosquinone could thus serve as a structurally relevant scaffold for future optimization studies aimed at improving the engagement of the hinge region and CNS penetrance for therapeutic development in GBM.

Keywords— EGFR, Glioblastoma, Diosquinone, Erlotinib, Molecular Docking, ADME, in-silico analysis.

1. Introduction

1.1 Glioblastoma Overview

Glioblastoma was ranked by WHO as an aggressive Grade IV astrocytoma on the basis of its acute proliferation, infiltrative growth pattern and immune-suppression of traditional therapeutic methodologies [1]. Regardless of advancements in treatment procedures, life expectancy remains poor, with limited clinical outcome even under current therapeutic protocols [2]. Because of its ability to scatter and diffuse rather than as a single solid mass, total surgical excision is marginally feasible, leading to unavoidable recurrence of the disease [3].

The current operative framework for this type of disease involves surgical excision sequentially by radiation and chemotherapy, predominantly by temozolomide [4]. Despite these combinatorial approaches, patient therapeutic efficacy remains limited, exhibited a median overall survival by roughly 14-16 months [3]. The limitations of these currently available therapies are mainly due to tumor heterogeneity, acquired resistance mechanisms, and the selective permeability of the blood-brain barrier, which obstructs drug delivery to the tumor site [5]. Therefore, these challenges underscore the development of innovative therapeutic strategies that can resolve these multifaceted challenges.

1.2 Epidermal Growth Factor Receptor Role in Glioblastoma

EGFR signaling is a transmembrane receptor tyrosine kinase, which is important for cell growth, expansion, malignant proliferation, differentiation and survival [6]. In glioblastoma, the mutation or amplification is often associated with EGFR which leads to biological downstream signaling pathways being stimulated, thereby promoting the specific biological forces responsible for the growth of the cancer [7].

The most common and most impactful alterations seen in EGFRvIII are due to its ligand-independent activity [7]. The oncogenic signaling is responsible for the major tumorigenic pathways including PI3K/AKT and MAPK, which accelerate unchecked growth, induce hyper-angiogenesis and inhibit apoptosis [8]. These signals are constantly activated and the tumor is highly invasive and difficult to treat and is correlated with poor clinical outcomes [9].

Due to its fundamental mechanism involved in malignant phenotype, EGFR has emerged as an important target for therapeutic targeted strategies [10]. Interestingly, the ability of the inhibitor to inhibit the target kinase effectively is not only determined by a high binding affinity and accurate molecular interaction with the kinase domain to validate efficient disruption of downstream signaling pathways, but also by the ability to inhibit the enzyme in its active conformation [11].

1.3 Precision Therapeutic Targeting of EGFR and Its Limitations

Selective inhibitors of the ATP binding site of EGFR have been designed and formulated as TKIs thus blocking the receptor activation [12]. The first-generation inhibitor like erlotinib binds competitively binds within the catalytic site [13].

Although clinical response has been seen in certain malignancies, their use in glioblastoma has been shown to be less effective and generally unsuccessful [14]. The BBB is one of the greatest challenges and reduces clinical effectiveness in cancer [15]. The compounds even having a favorable binding characteristic, may still fail to reach enough concentration levels within the brain tumors [16]. In addition to providing problems, glioblastoma may develop intrinsic resistance or develop resistance over time to targeted therapies [17]. Furthermore, the drug's transport through the body is hampered by problems such as solubility and metabolic variability, which can substantially reduce its therapeutic effectiveness [18]. All these together, emphasize that a better choice of approaches is necessary, that is, ones that are able to truly incorporate molecular target with better delivery strategies.

1.4 Natural Compounds as a Promising Source of Novel EGFR Inhibitors

Due to their chemical diversity and broad range of their biological activities, natural compounds are becoming a promising source for drug discovery [19]. Compared to developed synthetic compounds, which are specifically engineered to act on a single target, natural compounds interact with several pathways and may have pleotropic effects in complex diseases like glioblastoma [19]. For example, flavonoids, terpenoids, polyphenols and xanthones are among the classes of natural compounds that have been found to exhibit potent anti-proliferative, anti-angiogenic, and pro-apoptotic properties in this type of malignant cancer [20], [21]. Due to their structural diversity, they have the potential to interact with their targets in multiple ways, making them promising candidates to overcome resistance mechanisms of conventional treatments [21]. In EGFR targeting, natural compounds provide a new type of binding structures that differs from conventional ATP-competitive inhibitors [22]. Despite their potential, however, systematic investigation of their binding and in vivo effects find difficulty to go beyond the docking score-based methods of evaluation.

1.5 In-silico Approaches in Modern Drug Discovery

The recent in-silico biological innovation have transformed the early drug process by enabling high throughput and cost-effective screening of potential therapeutic compounds [23]. Computational methods like molecular docking and pharmacokinetic predictions, helping evaluate ligand binding and how suitable it binds prior to the experimental lab testing [23].

It offers detailed insights into various interaction types such as hydrogen bonding, hydrophobic interactions and electrostatic forces [24]. Emphasizing how the need for binding mode analysis to ensure meaningful interpretation [25].

At the same time ADME characterization plays an integral role in assessing pharmacokinetic behaviour and overall drug-likeness [24]. The key factors such as LogP, TPSA and molecular weight plays a critical roles of a selected compounds and their pharmacokinetic stability [24].

This combination application of molecular docking and ADME profiling establishes a robust framework for identifying and prioritizing the promising drug candidates in the early stage drug discovery [23].

1.6 Aim and Objectives of This Study

This present study seeks to evaluate binding affinity binding mode differences of natural compounds as a promising EGFR inhibitor using a structural based computational framework. Erlotinib was utilized as reference ligand to provide a benchmark to compare the binding affinities and interaction patters.

2. Literature Review

2.1 Glioblastoma: Molecular mechanisms and Therapeutic Limitations

Glioblastoma is a very aggressive, heterogeneous brain tumour, with several abnormalities at the genetic and molecular level. The tumor is heterogeneous and constantly changes in response to treatment stress, making therapy management challenging. The key takeaway is that it is not a uniform disease but a highly heterogenous tumor system, meaning the diverse tumor system is made up of different types of cells that coexist and dynamically evolve by reacting under treatment stress [26].

The ability of glioblastoma to change in the tumor environment is the key reason why it is highly aggressive. The tumor is characterized by hypervascularization due to the excessive production of blood vessels by VEGF, which facilitates nutrient distribution and excretion in the tumor that in turn allows for the quick proliferation of tumor cells [27]. These blood vessels however are abnormal and will not form optimally, due to its inability to deliver oxygen and will result in a hypoxic condition in the tumor region. This hypoxia also contributes to increased invasiveness of the tumor, which is achieved by the activation of HIFs, leading to the transcription of genes involved in cell growth, Angiogenesis and metabolic shift [28].

In addition to vascular abnormalities, there are subpopulations of glioblastoma stem cells (GSCs) as well. This is an important cell that is essential to the maintenance and recurrence of tumors. These cells have the ability to renew themselves and can exhibit more resistance to traditional strategies like chemotherapy and radiotherapy. They are able to repopulate the tumour after treatment which is a significant contributor to the disease relapse and progression, and to therapeutic failure [29].

In current treatment methods for glioblastoma involves a multimodal intervention, it involves removal of tumor by maximum surgical excision followed by chemotherapy and radiotherapy usually with temozolomide. Even though this method offers temporary control the disease, but this is not effective enough to suppress the recurrence of the tumor cells due to its persistent cancer cells and its inability to eliminate infiltrative disease [30]. Furthermore, over time glioblastoma has inherent and acquired resistance to therapy, as a result of its genetic changes and its surrounding tumor microenvironment.

Despite a broad and comprehensive research, the existing therapeutic approaches focus on the symptoms and do not cure the disease. Because of its complexity such as tumor diversity, invasiveness, hypoxic microenvironments, and impedes drug delivery across the BBB, emphasized the need for the development of promising, molecular mechanism involved in progressive disease.

2.2. EGFR in Glioblastoma: Structural and Signaling Aspect

EGFR, structurally composed of an outer part that has a ligand binding domain, a single transmembrane part that transverse the cell membrane, and an inner region with intracellular tyrosine kinase domain that activates downstream signaling. When a ligand binds to EGFR, it goes under dimerization and is succeeded by autophosphorylation of specific tyrosine kinase residues within the intracellular region, thereby triggering multiple signaling cascades that maintains the heterogeneity responses inside the cell [31].

In glioblastoma, EGFR is one of the most frequently observed by increasing copy number with amplification and mutational changes occurring in major part of the substantial proportion. Among these mutations, EGFRvIII mutation is the most common prevalent variant, characterized by a deletion within the extracellular domain that confers ligand independent that makes the receptor always active, even without a signal [8]. This constant activation drives the continuous downstream signaling leading to the uncontrolled cell proliferation, enhanced survival and more aggressive tumor behaviour.

- **PI3K/AKT/mTOR** pathway: This pathway initiation is triggered by the activation of the EGFR, which in turn helps the cells to grow, survive and metabolism. The activation of PI3K leads to the synthesis of phosphoinositide intermediates that attracts and activates AKT, which then chronologically regulates the downstream processes such as protein production and suppress the programmed cell death. In this particular tumor, this pathway is often hyperactivated due to EGFR amplification or loss of the cancer suppressor PTEN, leading to persistent cell proliferation and enhanced acquired resistance to survive treatments that earlier would have inhibit them [17].
- **MAPK** pathway: At the same time, EGFR activation would also engage with the MAPK cascade, which regulates cell growth, differentiation and gene expression. The process follows the “top-down” activation of RAS, RAF, MEK, and ERK, leading to the transcription of genes that makes the tumor more aggressive and is strongly related with resistance formation against therapeutic interventions [26].

Apart from the main signaling pathways, EGFR activation also engages with additionally regulatory networks that regulates cellular processes such as blood vessel formation, cellular invasion and metabolic reprogramming. The functional interplay between EGFR with other receptor tyrosine kinase makes the oncogenic signals stronger, allowing the malignant cells to sustain growth even with targeted treatments to block EGFR. This significant signaling redundancy within signaling networks represents a major challenge in attaining a durable therapeutic outcome.

Additional structural characterization of the EGFR kinase domain:

EGFR kinase exhibits a high conserved structure in ATP binding pocket and consists of different subdomains, including the hinge region, the catalytic loop, glycine-rich loop and the activation loop. Of all these, the hinge region especially forms crucial hydrogen bonds with ATP and ATP competitive inhibitors, thereby stabilizing ligand binding and stable orientation within the active site [31], [32].

In addition to the hinge area interaction, other structural features such as the hydrophobic pocket and neighbouring residues, helps in ligand stabilization through non-covalent interactions. Any variations in these interactions can significantly impact an inhibitor selectivity and its ability of inhibition. Therefore, in depth understanding of the structural

determinants for effective binding within the EGFR kinase domain is needed for designing and reviewing of promising inhibitors [32].

Overall, the fundamental role of EGFR in glioblastoma pathogenesis, combined with its structural features enable in targeted inhibition, highlighting its importance for a therapeutic target. Nevertheless, the complexity of its signaling and presence of constant active mutations, emphasizing the need for a deeper understanding of ligand-protein interactions beyond conventional inhibition methodologies.

2.3. Current Therapeutic Strategies Targeting EGFR Inhibitors and Limitations

Targeting EGFR has been an important strategy in glioblastoma due to its central role in tumor progression and survival. The small molecules TKIs are adapted to inhibit the EGFR activity in intrinsic tyrosine kinase by competitively binding it to the ATP- binding domain thereby inhibiting EGFR autophosphorylation and subsequently helps in blocking downstream signaling essential for tumor growth.

Efflux channels such as P-glycoprotein and ATP-binding cassette subfamily G member 2 diligently remove these drugs from the brain, further reducing concentrations inside the tumor [33]. Furthermore, the endogenous structural similarity of the EGFR ATP-binding pocket with other receptor tyrosine kinases often leads to off-target effects, impairing specificity and facilitates to a limited therapeutic values in glioblastoma [34]. These challenges are synthesized by the existence of both acquired and innate resistance mechanisms, such as secondary mutations in EGFR or activation by avoiding signaling pathways [35]. For example, erlotinib and gefitinib, while efficient in other malignancies, have exhibited limited clinical activity in glioblastoma clinical studies, predominantly due to poor blood-brain barrier penetration and export by active transporters [36]. However, resistance to EGFR TKIs in glioblastoma is not solely mediated to genetic mutations but can also emerge from adaptive cellular responses and activation of alternative bypass signaling pathways such as MET or PDGFR, thereby bypassing EGFR blockage and facilitating downstream signaling that is required for cellular survival and proliferation [37], [17].

Moreover, the tumor heterogeneity of EGFR expression and the presence of diverse EGFR mutations, the common EGFRvIII deletion, further hinder targeted therapeutic interventions in glioblastoma [38],[39]. Especially, their molecular weight, generally above 400, and electronegativity difference often limits in the adequate brain permeation, limiting their therapeutic potential regardless of promising in vitro expression [40]. This poor brain distribution considerably constraints their efficacy, as the blood-brain barrier prevents over 98% of small molecule drugs from penetrating inside CNS in sufficient concentrations to stop the tumor growth [41], [42].

All these limitations show that we need alternative solutions in therapeutic strategies beyond conventional small-molecule kinase inhibitors [43]. Therefore, studying different compounds and how their molecular binding interactions and pharmacokinetic profiles may provide promising therapeutic outcomes and overcoming resistance in glioblastoma.

2.4. Natural Compounds as A Potential EGFR Inhibitors In GBM

Unlike many conventional drugs that are typically designed to target a singular pathway, naturally derived compounds can exhibit pleiotropic behaviour, enabling them to regulate

complex and integrate signaling networks involved in tumor growth [20]. This property is particularly important in glioblastoma, where tumor diversity, adaptive strategy, and functional redundancy altogether reduce the therapeutic index.

The essential biological functions of natural compounds in glioblastoma consists of:

- **Growth inhibitory effects:** suppression of dysregulated cell divisions by controlling the key cell cycle regulatory proteins.
- **Devascularization activity:** prevents tumor vascularization through the down regulation of pro angiogenic factors such as VEGF.
- **Triggering of programmed cell death:** initiation of apoptosis mediated by mitochondrial and the activation of the executioner enzymes or caspase dependent signaling pathways.
- **Attenuation of oncogenic signaling:** regulation and interference of key signaling pathways such as EGFR, PI3K/Akt/mTOR, and MAPK which are frequently overexpressed in GBM.

The main polyphenol found in *Curcuma longa*, curcumin, has been demonstrated to inhibit glioblastoma cell proliferation by simultaneous inhibition of the EGFR phosphorylation, nuclear translocation of NF-κB and induction of autophagic cell death [44]. Its poor aqueous solubility, however, and high glucuronidation rate in the liver result in low systemic bioavailability and are the reason behind growing interest in nano-formulation approaches. Likewise, resveratrol (3,4′,5-trihydroxystilbene) has blood-brain barrier permeability that is relatively better and has been shown to sensitize glioma stem-like cells to temozolomide and to inhibit the PI3K/AKT signaling pathways [45], [46].

Quercetin is able to cause dose-dependent apoptosis in glioblastoma cell lines, involving the activation of caspase-3 and the reduction in the expression of Bcl-2. EGCG, an isolated catechin from green tea, has also been reported to competitively bind the ATP-binding domain of EGFR kinase with low micromolar level of inhibition [47].

An example of less-studied natural scaffolds is the Withaferin A present in *Withania somnifera*, which can cause oxidative stress and alter the organization of the cytoskeleton in glioblastoma cells via the vimentin pathway [48] . Similarly, α-mangostin from *Garcinia mangostana* has exhibited anti-proliferative activity in glioma cells derived from patients and has inhibited the autophosphorylation of EGFR [49] .

Compounds derived from medicinal plants have shown promising efficacy through multifunctional mechanisms, accounting to competitive binding within the ATP-binding site of EGFR, thereby optimizing its signaling [50]. These findings suggests that natural compounds have the therapeutic potential to not directly block but also affects the regulation of multiple cancer related pathways.

One major advantage of the natural-product-based libraries is that they are more structurally diverse than the usual synthetic kinase inhibitor collections. Natural compounds tend to be more stereo chemically complex, to have fused ring systems, and a greater number of sp^3-hybridized carbons, which allow targeting of biologically relevant regions of chemical space with improved target selectivity [51]. However, some natural compounds, especially polyphenols, might demonstrate PAINS properties or metal-chelating activity, leading to false positive screening results [52].

Comparative structural advantages of natural compounds compared to classical inhibitors:

Natural compounds are characterized with structural complexity and chemical diversity, which can facilitate novel binding modes with protein targets, potentially leading to improve specificity and reduced off-target effects as compared to synthetic inhibitors [53], [54]. Moreover, their intrinsic biocompatibility often leads to improved pharmacokinetic profiles and reduced systemic toxicity, contributing to the potential as therapeutic agents in glioblastoma treatment [55]. This structural diversity enables them to adopt multiple conformations and engage with a broader range of target protein pockets, including those considered "undruggable" by conventional small molecules.

As compared to the classical EGFR inhibitors which predominantly depend on hydrogen bonding with hinge region residues for stabilization, natural compounds may demonstrate diverse and alternative interactions such as:

- **Hydrophobic interactions:** involving non-polar residues within the binding pocket, contributing to overall binding affinity and stability [56] .
- **π–π stacking interactions:** occur between the aromatic rings of ligands and specific amino acid residues, particularly those with aromatic side chains like phenylalanine, tyrosine, or tryptophan [57].
- **Cation–π interactions:** electrostatic interactions between a cation and the positively charged residue (arginine) and which can significantly enhance binding specificity and potency [58].

These non-classical interactions can still show ligand stabilization even in the absence of strong hinge region hydrogen bonds. Such binding flexibility may enable natural compounds overcome resistance mechanisms coupled with traditional ATP-competitive inhibitors and to bind with underexplored regions of the active site.

Despite the growing attention in natural compounds, a major limitation is the predominant focus on docking scores alone to judge potential ligand potency. Important parameters such as interaction types and spatial orientation within the active site are often overlooked in this conventional virtual screening workflow [59] .

Pharmacokinetic Limitations in GBM

Beyond how well they bind, it is important to consider the clinical applicability of natural compounds and how strongly it is influenced by their pharmacokinetic characteristics particularly restricted BBB and high TPSA that often correlates with reduced cellular permeability, further impeding brain access [60].

Relevance of the present study

This study addresses these critical limitations by employing a comprehensive in silico approach that integrates molecular docking, protein-ligand interaction profiling, and ADME predictions. Instead of solely focusing on binding affinity, this study incorporates:

- Comparative analysis of binding modes and assess the presence of critical interactions, particularly within the hinge region of EGFR [59].

- Differentiation between classical hinge region hydrogen binding and alternative non-classical interactions for ligand stabilization [61].

This integrated approach allows for a more reliable identification of promising natural compounds that not only exhibit high affinity for EGFR but also possess favorable pharmacokinetic profiles crucial for glioblastoma treatment.

2.5 Computational Approaches for Structure Based Drug Discovery

Latest advancements in computational methodologies in biology have transformed the preliminary stages of drug identification by facilitating rapid, high- throughput and cost efficient virtual screening of potential pharmacological compounds [62]. These computational techniques enable the standardized assessment of molecular engagement between ligands and target proteins, prediction of binding affinities and evaluation of their ADME properties before experimental verification. Such methods are especially advantageous in complex diseases like glioblastoma, where conventional drug discovery pipelines are often resource-intensive, time-consuming and are often unsuccessful.

Molecular Docking and Binding Mode Analysis

Computational docking is one of the most widely used to estimate interactions of how a drug binds to a target protein and provides a scoring function indicating preferred binding orientations and binding affinities. However, docking scores solely do not always associate with biological activity, since they may not show the stability and functional importance of interactions [59].

Limitations of traditional docking protocols:

- Insufficient consideration of protein flexibility during ligand binding, can potentially lead to inaccurate binding pose predictions.
- Simplified process of solvent effects, which can influence the incomplete specification of binding pose.
- Over-dependence on scoring estimations as key drivers of binding affinity, without considering about biological efficacy.
- Inadequate focus on specific interactions.

Limitations of Docking-Based Predictions of EGFR inhibition activity

The docking simulation of the protein and ligand is a simplified representation of real biological conditions, and may not fully reflect the behaviour of the protein and the ligand within the cell. Movement of protein, solvent effects, flexibility of ligands are factors that can affect binding behaviour, but are only partially accounted for when making docking predictions [25].

Therefore, to mitigate these limitations, detailed binding mode analysis is essential for evaluating the nature and robustness of ligand-protein interactions within the active site. However, other interaction like non-classical interactions like hydrophobic contacts and π-based interactions, may also mediate significantly in ligand binding and should be carefully analysed.

Interaction Profiling and Structural Analysis

Irrespective of binding score functions, a thorough interaction profiling offers detailed insights into ligand-protein binding mechanisms. This approach includes the identification of specific molecular interactions like hydrogen bonds, hydrophobic contacts, π–π stacking, and cation-π interactions, as well as an assessment of their spatial arrangement within the binding pocket. Such analysis may be used to find out the most important amino acid residues for the stabilization of the ligand in the active site.

The fundamental factors governing binding effectiveness include:

- The overall stability of the ligand within the active region.
- Proper spatial positioning and orientation of the ligand.
- Significant role of non-classical mechanisms.

It is especially useful for comparing classical ATP-competitive inhibitors from compounds that interact through different interaction mechanisms.

Computational ADME Profiling and Pharmacokinetic Evaluation

In drug discovery, accurate prediction of a compound's Absorption, Distribution, Metabolism, and Excretion properties is essential for assessing its therapeutic potential and minimizing late-stage withdrawal. Therefore, plays a crucial role in filtering out compounds with suboptimal pharmacokinetic profiles early in the discovery pipeline, specifically for CNS-acting drugs where blood-brain barrier permeability is critical.

Key pharmacokinetic parameters include:

- **Lipophilicity (LogP):** determining membrane penetration and absorption properties.
- **Molecular weight:** influence the distribution, biological accessibility, and overall pharmacokinetic performance.
- **TPSA:** an important determinant of cellular permeability and blood-brain barrier transversion.
- **Solubility and stability:** govern the availability and the duration of the potential compounds.

The compounds that demonstrates favorable ADME properties and therefore more likely to achieve and attain effective therapeutic concentrations at the tumor region [16].

Integrated In Silico Framework for Drug Discovery

The integrated computational framework combines molecular docking, binding mode analysis, and ADME profiling to identify promising lead compounds for glioblastoma treatment. Rather than solely relying on docking scores, this methodology enables a more comprehensive assessment and holistic determination of both binding efficiency and ADME stability.

Within this approach, the current study adopts a combined computational strategy to evaluate natural compounds as promising inhibitors of EGFR in glioblastoma, highlighting on binding mode properties and pharmacokinetic stability. This study aims to address the limitations of

conventional screening methods and to offer a deeper understanding into ligand-targeted protein interactions relevant to therapeutic intervention in glioblastoma.

2.6 Research Gap and Purpose of the Study

Regardless of significant progress in understanding the hidden mechanisms of EGFR signaling and its implications in glioblastoma advancement, there are still major challenges that limit the development of robust targeted therapies.

In most in silico investigations, the detailed docking mode evaluation was not considered important, but primarily the docking scores are taken into consideration for the selection of the promising inhibitors.

Most EGFR inhibitors are designed based on the classical ATP-competitive binding involving hydrogen bonds on the hinge region. However, other interactions like hydrophobic contacts, π–π stacking and cation–π interactions are not well studied. These non-classical interactions might significantly aid in the stabilization of the ligand and contribute to overcoming the resistance mechanisms [63]. However, there are numerous in silico studies that estimate binding affinity but do not consider the pharmacokinetic properties in the effectiveness of therapies for glioblastoma.

There is a lack of good integration of structural interaction analysis with ADME profiling, which hampers the translation of computational prediction. This constraint hinders the discovery of compounds with not only high binding affinity, but also appropriate pharmacokinetic characteristics for delivery to the central nervous system.

These gaps indicate the need for a more sophisticated computational model that is beyond simple docking-only screening. To address this, this study adopted a molecular docking study along with detailed binding mode analysis and pharmacokinetic study.

This study also incorporates ADME profiling, specifically blood-brain barrier permeability, to provide a list of compounds that have favourable binding to the receptor and appropriate pharmacokinetic properties for use in glioblastoma therapy. Combining binding affinity, interaction specificity, and pharmacokinetic properties creates a more comprehensive model to choose candidate EGFR inhibitors.

The purpose of this study is to highlight various structurally rich naturally occurring compounds for the purpose of exploring novel molecular scaffolds to overcome the limitations of the existing synthetic inhibitors and provide superior therapeutic efficacy. The computational predictions can be used as a foundation for future experimental testing and rational drug design of glioblastoma.

3. Methodology

3.1 Research Design and Computational Framework

In silico drug discovery approach is used to assess the therapeutic potential of naturally occurring phytochemical compounds against EGFR tyrosine kinase domain associated with GBM. The in silico approaches provide a more efficient and less costly alternative to experimental testing to perform pre-selection of candidate molecules and generate hypotheses

systematically, rather than discard or allow molecules to drop out at later stages of the drug discovery process [64].

The entire workflow includes four analytically integrated steps:

(i) retrieval and preparation of macromolecular target

(ii) curation and preparation of the ligand library

(iii) structure-based molecular docking and binding mode characterization

(iv) computational prediction of pharmacokinetic and drug-likeness properties

These stages are then integrated, allowing for a more comprehensive evaluation of candidate compounds than binding affinity alone, and also considering the quality of interaction and the pharmacokinetic properties of the compounds as co-determinants for therapeutic relevance.

3.2 Target Protein: Selection, Retrieval, and Structural Rationale

3.2.1 Biological Significance of EGFR

The intracellular kinase domain of EGFR contains a canonical small-molecule binding site called the ATP-binding pocket. The P-loop, the hinge region, the activation loop and the catalytic loop create the delineation of this pocket. The hydrogen bonding between the hinge region of EGFR in human residues around 793–796 and the N1 and N3 positions of adenine in ATP is mimicked by almost all clinically used TKIs of the first generation, which are derived from EGFR, and thus is a basic pharmacophoric feature [12]. It is in this region that the structural conservation was observed, and high-resolution crystal structures are available, making EGFR very suitable for structure-based computational screening.

3.2.2 Crystal Structure Selection

Three-dimensional crystal structure of the kinase domain of EGFR used in this study was obtained from RCSB Protein Data Bank (PDB 1M17) (PDB; https://www.rcsb.org/). The structure is that of the human EGFR kinase domain, residues 696-1022 bound to the inhibitor AQ4 resolved using X-ray crystallography at 2.60 Å resolution. The following PDB ID 1M17 was selected for the following reasons:

Structural completeness: The ATP-binding pocket of the asymmetric unit is basically complete with the hinge region, P-loop, catalytic loop and activation loop well resolved, allowing for accurate docking simulation.

Conformation with Ligand: The structure is the active (DFG-in) conformation of EGFR bound with Ligand. The screened natural compounds are expected to be ATP-competitive (Type I) inhibitors of the active conformer, which makes the structure a receptor model that is biologically appropriate.

Resolution suitability: A resolution of 2.60 Å is considered to be suitable for docking studies in which the structure is considered, and there is enough resolution to accurately position polar residues that are expected to participate in hydrogen bonding, but not so low as to be impractical for computational purposes.

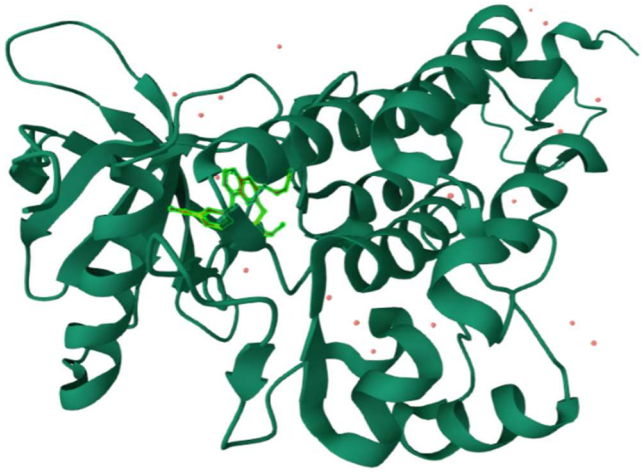

Fig.1. Human EGFR tyrosine kinase domain with 4-anilinoquinazoline inhibitor erlotinib

3.2.3 Protein Structure Preparation

The crystal structure of the EGFR tyrosine kinase 1M17 was chosen from the RCSB Protein Data Bank because it is the active kinase domain of the EGFR, co-crystallized at high resolution with the clinically used inhibitor erlotinib. The validated ATP-competitive inhibitor was located in the binding pocket and allowed the active site residues coordinates to be unambiguously identified and provided a structurally relevant reference for comparative docking analysis. Also, 1M17 is among the most widely used EGFR docking structures for use in computational inhibitor-screening studies, and can therefore be used for benchmarking the behaviour of binding of the ligands with the EGFR in terms of a known classical EGFR inhibitor binding mode.

ADT v1.5.7 in the MGLTools suite was used to prepare the protein [65] . A careful preparation is required to guarantee that the receptor model corresponds to a stable conformation suitable for docking simulation, which is physiologically stable. The next series of steps were carried out sequentially:

Removal of water molecules: All crystallographic water molecules found in PDB 1M17 were removed. Positional ambiguity of the active site and steric or electrostatic interference of the docking results are problems in rigid-body docking protocols like AutoDock Vina, where explicit water molecules are used. In structure based virtual screening, generally water molecules are omitted from the virtual screening process if they are not directly involved in critical interactions.

Removal of co-crystallized ligand and non-essential heteroatoms: non-essential heteroatoms in the protein, such as buffer ions, glycerol, DMSO artefacts were removed from the

coordinate file, as were the co-crystallized AQ4 molecules. If the co-crystallized ligand is retained, then the docked compounds will not be able to access the binding site.

Addition of Polar Hydrogen Atoms: Most crystallographic structures do not have hydrogen atoms in the crystal, since they are not seen at typical X-ray crystallography resolution. The protein structure was protonated as per the automated protonation module in ADT.

Assignment of Gasteiger partial charges: All protein atoms were assigned Gasteiger–Marsili charges, through iterative PEOE method. These are empirical partial atomic charges which are used to approximate the electrostatic potential surface of the receptor and to assess electrostatic contributions to binding by the AutoDock Vina's scoring function. Gasteiger charges provide a good approximation in terms of speed and accuracy for use with high-throughput docking procedures.

Format conversion to PDBQT: The fully prepared protein structure was saved in the PDBQT format, which contains atomic coordinates, Gasteiger partial charges (Q) and AutoDock atom types (T) within a modified PDB file. PDBQT is the format needed for AutoDock Vina, and contains all the parameters used in docking simulation.

3.3 Grid Box Definition and Active Site Delineation

One important factor in determining the reliability of the docking is the definition of the docking search space known as grid box, which limits the conformational sampling of the ligand in the search process to the biologically relevant binding site [66]. In this study the grid box was centered on the centroid of the ATP-binding pocket was selected for the grid box, covering the hinge, P-loop, catalytic loop and hydrophobic back pocket. The grid spacing was chosen to be 1.0 Å, which is typical for a simulation conducted in AutoDock Vina. All of the grid box parameter such as center coordinates X: 23.56, Y: 9.824 and Z:25.39 including the dimensions X: 25 Å, Y: 25 Å and Z:25 Å were specified in the docking execution configuration file (.conf).

3.4 Ligand Library Curation and Selection Criteria

A set of structurally diverse natural compounds was selected and was used for virtual screening. The compounds were chosen from a preliminary survey of the literature, focusing on phytochemicals that have been previously reported on as having anti-proliferative, pro-apoptotic, or kinase-inhibitory effects in the context of cancer [67]. These compounds were also being investigated for involvement in EGFR-mediated or related receptor tyrosine kinase signaling pathways. The following selection criteria were used including previous proof of activity in cancer cell lines or animal models, not necessarily against EGFR and structural features like phenolic scaffolds, quinone scaffold, flavonoid interaction was expected to show molecular complementarity to the hydrophobic and polar features of the EGFR ATP binding pocket.

The three-dimensional structure files (SDF) of each compound were downloaded from the PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/) which is the standardized and curated database of chemical structures derived from deposited experimental data and the library of choice for many in silico drug discovery workflows. To guarantee consistency in the preparation pipeline, the reference inhibitor erlotinib (PubChem CID: 176870) was also downloaded from PubChem through the same approach.

3.5 Ligand Preparation

All the ligands, including the reference compound erlotinib, were prepared for docking with PyRx v0.8 [23], a virtual screening tool that combines AutoDock Vina with an embedded energy minimization module. The preparation of the ligands involved the following steps:

3D structure importation: The SDF files downloaded were imported to PyRx. PyRx also has built-in Open Babel chemistry toolkit which was used to generate 3D coordinates for compounds that started in 2D format, using distance geometry algorithms that provided a reasonable initial structure.

Energy minimization: For each ligand, energy minimization was carried out using the UFF as part of the Open Babel module in PyRx. Minimisation is used to relieve any strained bond lengths, bond angles, or torsional angles that may have been generated during the 3D coordinate generation, to obtain a lowest energy starting conformation for docking.

Protonation and charge assignment: All the polar hydrogen atoms were added to each ligand and the partial charges of all the atoms of the ligands were assigned using the Open Babel implementation of Gasteiger partial charges within PyRx. The critical requirement for electrostatic interaction scoring with AutoDock Vina is that the charge is the same across both protein and ligand.

Flexible bond assignment: Bonds within each ligand which were able to be rotated were identified and flagged for conformational sampling during docking. AutoDock Vina uses a flexible ligand or rigid protein docking model in which the ligand is conformationally flexible, flexible all non-ring single bonds and a rigid protein receptor. This will preserve the conformational flexibility of the ligand, whilst keeping the calculations tractable and manageable.

Format conversion to PDBQT: Atom coordinates, partial charges and AutoDock atom type designations were added to each prepared ligand and it was converted to the PDBQT format.

3.6 Molecular Docking Protocol

3.6.1 Docking Engine: AutoDock Vina

The widely validated and open-source docking program AutoDock Vina v1.1.2, developed at The Scripps Research Institute, was used for molecular docking. AutoDock Vina uses an ILS gradient-based stochastic global optimization algorithm and a semi-empirical free energy scoring function based on the X-Score algorithm that includes terms for steric interaction like van der Waals forces, hydrogen bonding, electrostatics, hydrophobic effects, and torsional entropy penalty. The program concurrently optimizes the geometry of the ligand, its position and orientation in the search space for finding low-energy binding poses.

The use of AutoDock Vina over previous versions of AutoDock such as AutoDock 4 was justified by its computational speed due to multi-core parallelization, increased docking accuracy on standard datasets, and ease of integration with preparation pipelines (PyRx and ADT).

3.6.2 Docking Parameters

The next parameters were set in the AutoDock Vina configuration file:

Receptor file: Prepared PDBQT file of the apo form of EGFR (1M17). Ligand file: Individual PDBQT file for each screened compound.

Grid box center: Coordinates of the centroid of the reference ligand used that were interacting with the ATP-binding cavity.

Grid box dimensions: Set to cover entire ATP-binding cavity

Sampling: Set to 8 used as a default, which is a good compromise between sampling and computation speed for single-target docking.

Number of binding modes (num_modes): Which was set to 9, can generate up to 9 different binding modes for each ligand, and the best mode was chosen.

Energy range: The best or worst binding mode for the energy range holds that the maximum difference in energy between the best and worst binding mode is 3 kcal/mol.

All the compounds were individually docked to the EGFR receptor. The binding affinities were given in kcal/mole and more negative values were more strongly predicted. In order to achieve consistency, all docking runs were performed with the same configuration file.

3.6.3 Docking Protocol Analysis

The binding affinity and interaction modes of selected compounds in EGFR ATP-binding pocket were analysed by molecular docking studies using AutoDock Vina. Using a gradient-based conformational search algorithm and an empirical scoring function, AutoDock Vina determines the best possible conformation for a ligand and estimates the binding free energies in kcal/mol. A higher score of negative docking indicates greater predicted ligand binding affinity. A rigid receptor flexible docking approach was used for docking simulations. Several binding poses for each ligand were generated and the highest ranked binding pose was chosen for subsequent interaction analysis. Structural and pharmacokinetic studies were conducted on a subset of compounds with docking affinities similar to and or superior to that of the reference inhibitor erlotinib.

3.7 Protein-Ligand Interaction Analysis

3.7.1 Structural Visualization

The protein–ligand complexes attached to the docked structures were analysed in PyMOL (https://www.pymol.org/) to gain insight into the orientation and occupancy of a ligand in the ATP-binding domain of EGFR.

Comparative visualization between erlotinib and the natural compounds screened was carried out to look for differences in binding geometry and patterns of stabilization in the catalytic pocket.

3.7.2 Interaction Profiling

To identify the residue-level interactions between ligands and EGFR, detailed interaction analysis was performed in PlexView [68] .

The following were included in the interaction analysis such as hydrogen bonding interactions, hydrophobic contacts, cation–π interactions, van der Waals interactions and electrostatic interactions. The hinge region residues of EGFR were studied in detail, because they are important for the stabilization of ATP-competitive kinase inhibitors. Based on interaction patterns, ligands were comparatively classified into:

Classical hinge-region binding interactions and classical alternative stabilization mechanism. Other than the docking affinity, this distinction has been employed to assess the potential biological importance of ligand binding.

3.7.3 ADME and Pharmacokinetic Profiling

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The shortlisted compounds were subjected to drug likeness and pharmacokinetic properties evaluation using the SwissADME Web Tool.

The following parameters were studied such as gastrointestinal absorption, blood–brain barrier permeability, molecular weight, LogP, Gasteiger's TPSA, Lipinski's Rule of Five compliance, and bioavailability score.

Oral drug-likeness properties related to membrane permeability and absorption were assessed using Lipinski's Rule of Five. The permeability of BBB was of special interest since, for glioblastoma therapy to be effective, there must be good penetration into the central nervous system. The TPSA and LogP values were also evaluated to evaluate the membrane permeability properties and CNS accessibility of the tested compounds.

3.8 Software and Tools Summary

Table 1: List of all the software and tools used

Tool / Resource	Version / URL	Application
RCSB Protein Data Bank	PDB ID: 1M17	EGFR crystal structure retrieval (PDB: 1M17)
AutoDock Tools	v1.5.7 (MGLTools)	Protein preparation, PDBQT conversion
PubChem Compound	https://pubchem.ncbi.nlm.nih.gov/	Ligand structure retrieval (SDF/SMILES)
PyRx	v0.8	Ligand energy minimisation, PDBQT conversion
AutoDock Vina	v1.1.2	Molecular docking simulations
PyMOL	v2.x (Schrödinger)	3D visualisation of docked complexes
PlexView	Torrens-Fontanals et al., 2024	2D interaction profiling
SwissADME	https://www.swissadme.ch/	ADME and drug-likeness prediction

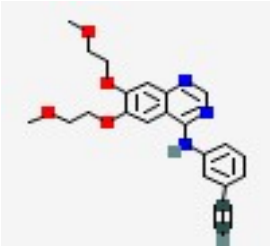
4. Results

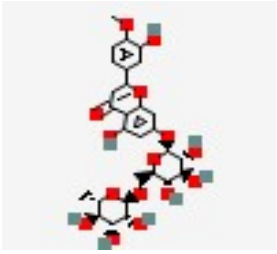
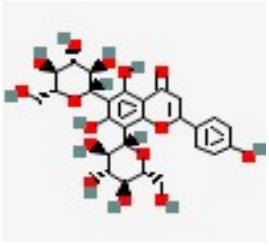
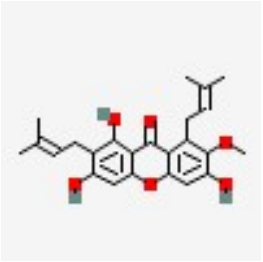
4.1 Molecular Docking Analysis of Natural Compounds Against EGFR

The first generation, EGFR tyrosine kinase inhibitor (TKI) Erlotinib, which has been approved for the treatment of non-small cell lung carcinoma was used as the reference compound because of its well characterized interaction geometry in the EGFR ATP-binding cavity [69].

Compared to all the natural compounds screened, diosquinone showed the highest predicted binding affinity (docking score of –10.1 kcal/mol) while erlotinib showed the lowest (docking score of –7.2 kcal/mol). Other compounds such as diosmin (–9.6 kcal/mol), vicenin-2 (–9.4 kcal/mol) and gamma-mangostin (–9.1 kcal/mol) also showed good binding abilities in the EGFR kinase domain. The totality of these results showed that several naturally occurring compounds were structurally compatible with the ATP binding site of EGFR. All the tested compounds are docked with the receptor to summarize the docking affinity values and even their 2D interaction compounds are shown in (Table II).

Table 2: Predicted docking affinities of selected natural compounds and the reference inhibitor erlotinib against the EGFR ATP-binding domain with their 2D structures and interaction residues.

Compound name	2D structural figure	Docking affinity (kcal/mol)	Interacting residues
Erlotinib (reference)		-7.2	ASP813, GLU738
Diosquinone		-10.1	ARG817, PHE699

Diosmin		-9.6	ASP776, ASN818, ASP831
Vicenin-2		-9.4	ASP776, ASN818
γ mangostin		-9.1	GLU738, THR766

Despite the good docking affinities of gamma-mangostin, diosmin and vicenin-2, interaction analysis indicated that diosquinone was a potential compound that had a different interaction geometry and showed comparatively higher affinity for the receptor.

4.2 Interaction Analysis of Erlotinib

Residue-level interaction profiling was performed to identify the molecular interactions that are involved in binding [70]

Erlotinib showed the typical interaction pattern of a competitive inhibitor of the EGFR, the adenosine 5'-triphosphate receptor. The stabilising interactions that the ligand formed with, specifically with ASP813, which fundamentally signifies the stabilization of the ligand within the active site. Beyond hydrogen bonding, a salt bridge interaction was observed with the same residues, elevating binding stability. The neighboring residues such as LYS721, GLU762, and LEU residues contributes in hydrophobic and electrostatic stabilization, ensuring greater stabilization was observed in the ATP-binding cavity as illustrated in the Fig.1. These were found to be of the classical ATP-competitive binding mode of EGFR

inhibitors and thus were used as reference for comparison with the natural compounds screened.

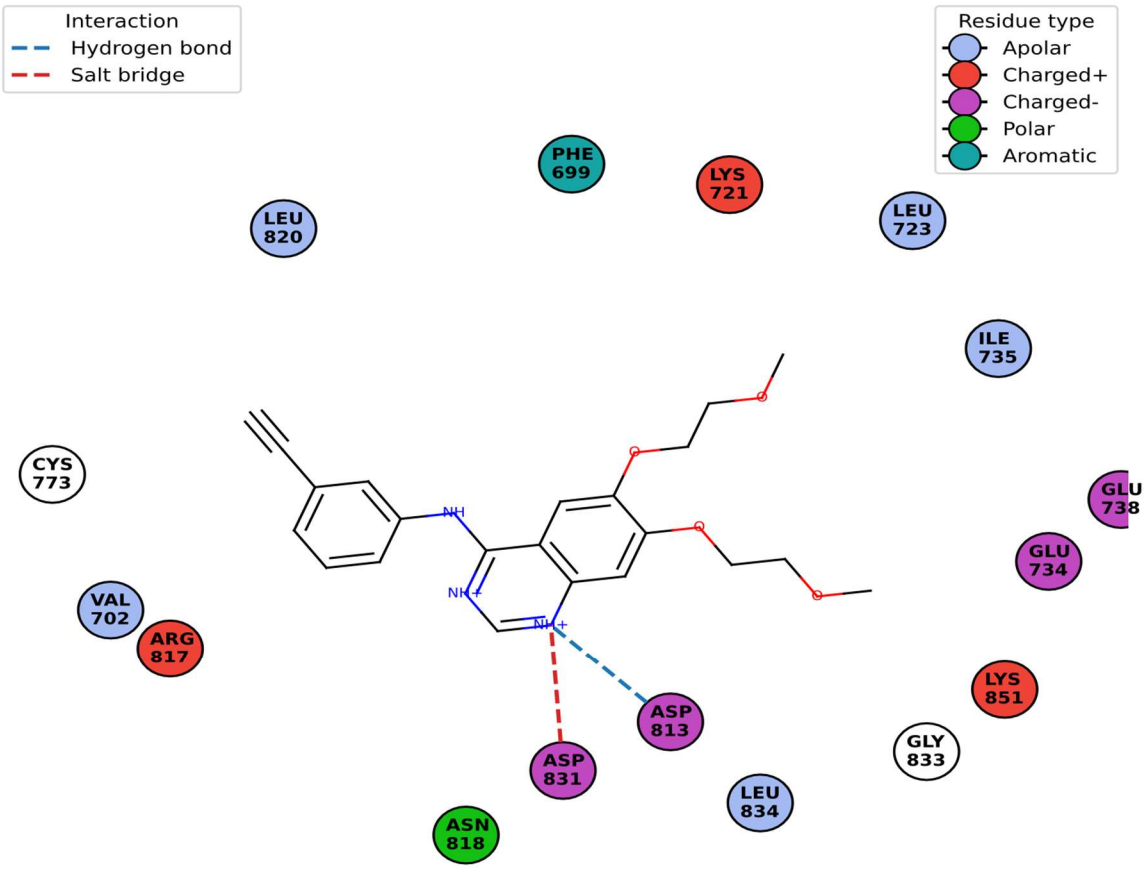


Fig. 1 Interactions of erlotinib

4.3 Interaction Analysis of Diosquinone

Among the natural compounds tested, the docking score for diosquinone was –10.1 kcal/mol, which is the highest predicted binding affinity. This binding energy was very favourable; however, residue-level interaction analysis showed a different binding mode than the classic EGFR inhibitor binding mode as shown for erlotinib. In particular, diosquinone was not observed to involve in the hydrogen bonding interactions with residues that are typically seen in classical EGFR ATP-competitive inhibitors. Rather, the stabilization of the ligand in the ATP-binding cavity seemed to be largely due to non-covalent, non-hydrogen bond interactions with amino acids in the periphery of the canonical hinge region and to cation–π interactions [71] as shown in Fig.2. The high binding affinity predicted for diosquinone indicated that classical hinge-region engagement was not always associated with high binding affinity. Instead, the compound was found to bind in an alternate conformation within the ATP-binding pocket, perhaps with different contacts to residues.

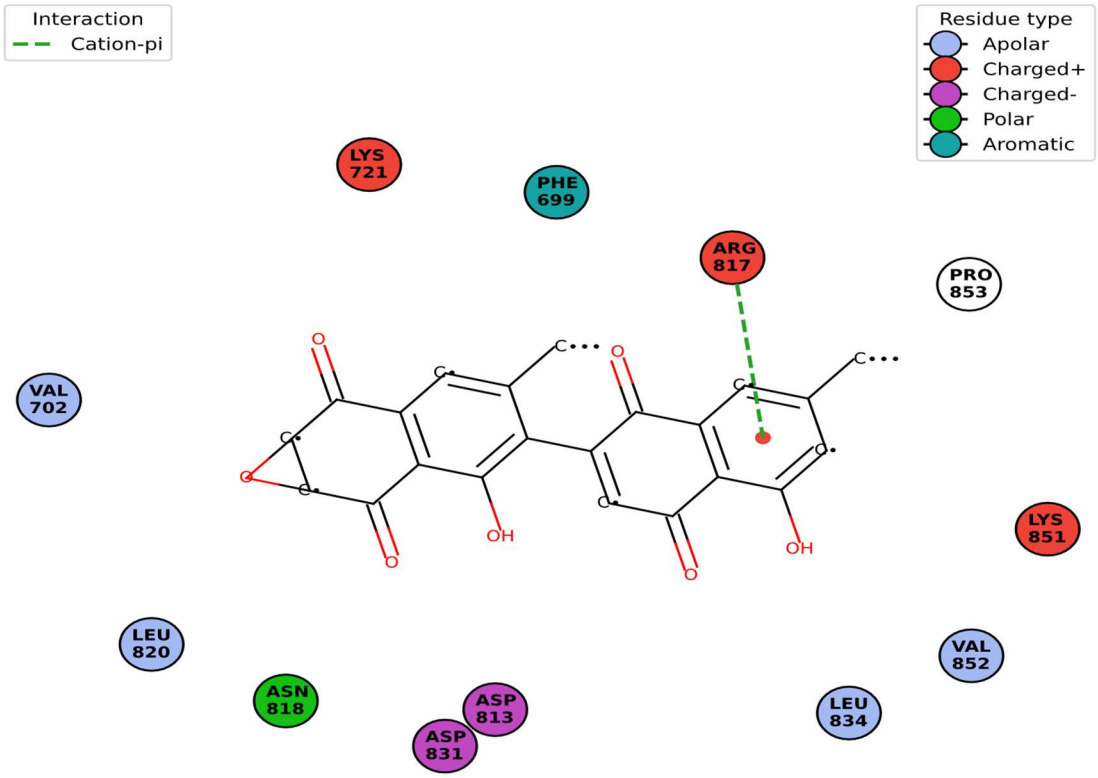


Fig. 2 Interactions with diosquinone

4.4 Comparative Binding Mode Analysis of Erlotinib and Diosquinone

Erlotinib showed the canonical interaction profile for ATP-competitive EGFR inhibitors, including hydrogen bonding interactions with the hinge region of the receptor, similar to existing experimental and computational studies. Although the binding affinity of Diosquinone was predicted to be good, it was found to have a non-classical binding profile with no canonical hydrogen bonding interactions in the hinge region of the receptor. The observations suggest that there are different structural modes of interaction with the ATP binding cavity of the EGFR that stabilizes a ligand. This integrative approach allows for more selective prioritization of downstream biological evaluation of candidate molecules [64].

The observed cation–π-interaction with ARG817 can be of importance for the electrostatic stabilization of the ligand in the binding pocket, in particular. The different binding orientation of erlotinib and diosquinone also highlights the significance of consideration of the different ligand positioning instead of just docking affinity values. The orientation of

erlotinib is classically the ATP-competitive one, whereas diosquinone seems to be in the binding cavity by another conformational arrangement. The dynamics of the receptors and the efficacy of the inhibitors in the downstream when in an in vivo environment.

Overall, these results suggest that high predicted binding affinity does not always equate with an optimum inhibitory nature for targeting EGFR in therapeutic interventions. Rather, detailed structural interaction profiling is needed to separate compounds that have structurally relevant binding properties from those that have energetically favorable, but biologically unclear binding properties.

4.5 ADME and Pharmacokinetic Profiling

No violations of Lipinski's Rule of Five were observed for both erlotinib and diosquinone, suggesting that both compounds have physicochemical properties with wide ranges of oral bioavailability. Molecular weight and LogP of Erlotinib were determined as 393.44 g/mol and 3.20, respectively, which are similar to those of clinically approved oral TKI. The molecular weight of Diosquinone was 284.26 g/mol, a relatively moderate number, and its LogP was 2.37, also a relatively moderate number.

Diosquinone has a higher TPSA value 121.27 Å² than the commonly used CNS-penetration cut-off of 90 Å². The results highlighted the importance of receptor-binding affinity in highlighting the potential therapeutic value of diosquinone in GBM, and the need for pharmacokinetic optimization, specifically in terms of CNS penetration, in the next steps of developing the drug as a candidate. The ADME profiles of both compounds have been summarized in Table 3, and SwissADME BOILED-Egg prediction model has been presented in Fig.3.

Table 3. Tabular representation of ADME and drug likeness foe erlotinib and diosquinone.

Parameters	Erlotinib	Diosquinone
Molecular Weight (g/mol)	393.44	284.26
LogP	3.20	2.37
TPSA (Å²)	74.73	121.27
GI Absorption	High	High
BBB Permeability	High	Limited

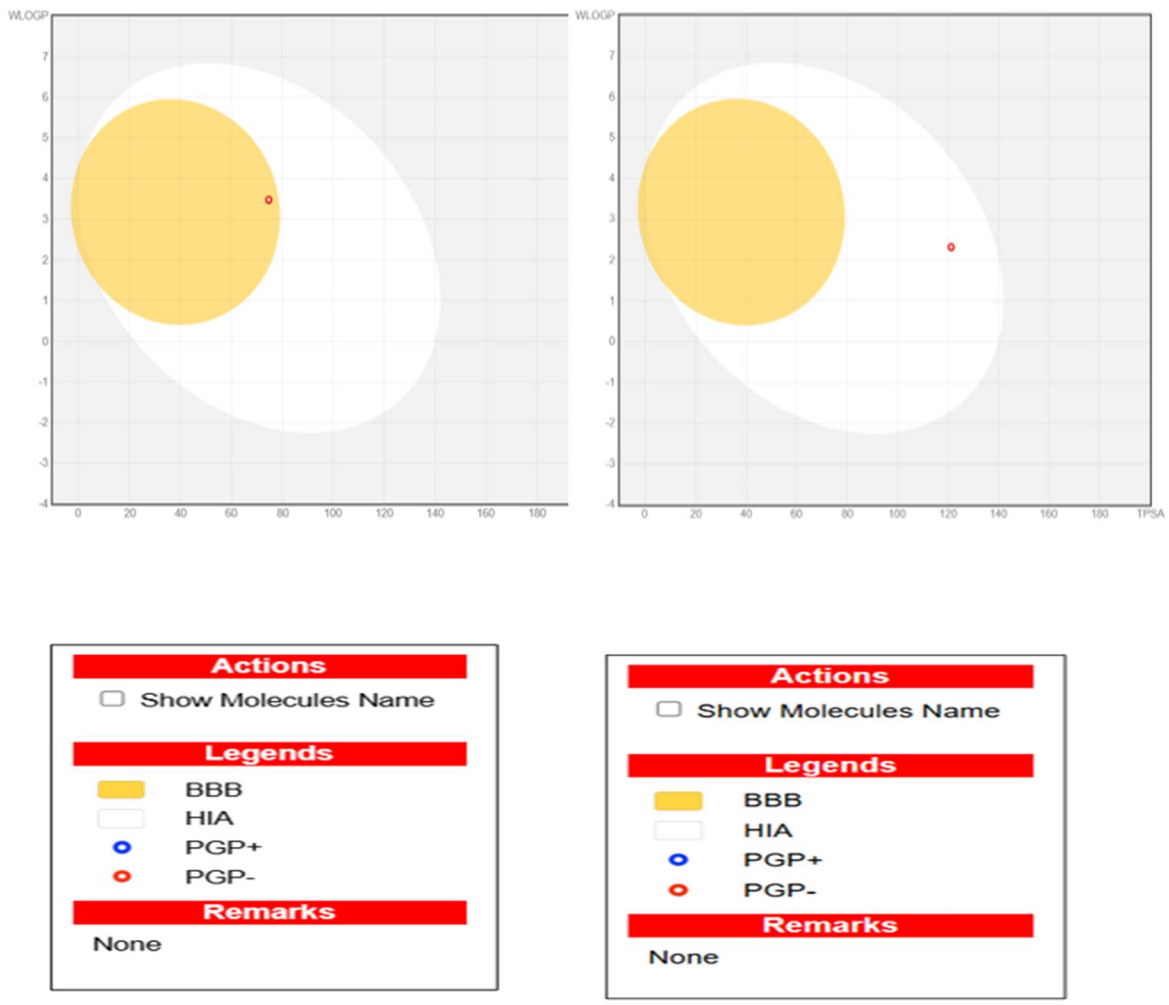


Fig. 3 SwissADME BOILED-Egg prediction model

5. Discussion

5.1 Docking Affinity: Interpretation and Limits

Of all the compounds tested, diosquinone showed the greatest predicted binding affinity −10.1 kcal/mol, significantly larger than that of erlotinib −7.2 kcal/mol, and the other compounds that were found to be in better predicted binding affinity within the ATP-binding cleft. The findings suggest that various chemotypes of natural products can be structurally accommodated in the EGFR kinase domain. But a docking score alone is not sufficient and necessary evidence of biological activity. It only approximates binding energetics without considering receptor flexibility, dynamic solvation effects, and most importantly, it is not possible to describe where and how a ligand is stabilized within the binding site [64]. This distinction is important when considering diosquinone, for which the affinity rank is not in line with the interaction profile.

5.2 Erlotinib as the Classical Binding Reference

Erlotinib is the Classical Binding Reference. Erlotinib showed the typical interaction profile of a competitive inhibitor of the EGFR. Within the ATP-binding pocket, its stabilization involved hydrogen bonding with ASP813, electrostatic interactions with LYS721 and hydrophobic interactions with GLU762, which is similar to the binding geometry [72] . Erlotinib interacted primarily with the hinge region, the part between the N- and C-terminal lobes of the kinase domain, through direct hydrogen-bond interaction with adenine, which mimics the interactions of ATP with the hinge region. The defining characteristic of the classical EGFR TKIs is an engagement in the hinge region, which was the structural model used when measuring the natural compounds.

5.3 Diosquinone: Strong Affinity, Non-Classical Interaction Profile

The main conclusion of this study is the seemingly paradoxical absence of classical EGFR binding behaviour despite the high predicted affinity of diosquinone. Although docking score for diosquinone was the highest, it did not make typical hydrogen bonds with the hinge region. Rather, its stabilization in the ATP-binding pocket seemed to be mainly mediated by cation–π interactions with side chains that aren't directly involved in the hinge interactions of erlotinib.

This is an important difference in terms of pharmacology. However, the contribution of cation–π interactions to the binding energy can be significant [73], and a compound without hinge-region interaction may not be able to effectively compete with ATP or may bind so that it is not a productive inhibitor of kinase activity. In the context of only a computational study, this implies that diosquinone is not only not considered a classical EGFR inhibitor, but that it is not classified as such based on its docking profile alone. It acts in a non-classical manner and its real mechanism of action like inhibitory, partial or even non-inhibitory is not known and yet to be confirmed at the experimental level.

This also highlights the importance of evaluating the interactions that occur at the residue level in addition to docking scores. The two compounds tested here make it abundantly clear that a similar, or better, affinity value can be obtained from a mechanistically different binding event. Use of a pharmacologically more meaningful prioritization scheme, based on the interaction profile of the compounds, classical hinge-engaging vs. non-classical peripheral, is more comprehensive than energetic ranking.

5.4 Pharmacokinetic Profiling and BBB Permeability

The erlotinib and diosquinone both met Lipinski's Rule of Five, thus indicating that there are no gross physicochemical barriers to oral bioavailability from either compound. But there was a difference of more therapeutic importance in their predicted BBB permeability. Physicochemical characteristics are suitable for Erlotinib to penetrate the CNS, this is relevant due to its investigation in brain tumor settings [74]. In contrast, Diosquinone was poorly predicted to penetrate the BBB with a TPSA of 121.27 Å², which is quite above the empirical ~90 Å² cut-off value after which passive transcellular diffusion across the BBB is significantly lower. This is validated by its status as not being in the brain-penetrant zone in the SwissADME BOILED-Egg model. In GBM, this is not a secondary barrier as it is with BBB absent, but a primary pharmacokinetic barrier in cases of BBB breach or dysregulation. If a compound can't get to the tumor site at relevant concentrations, it can't have a therapeutic effect, no matter how good its target affinity is [75]. In light of the docking results for diosquinone, it is therefore concluded that the TPSA finding supports the favorable docking

results of diosquinone and suggests that CNS penetrance is important parameter that needs to be optimized for any follow-up work.

5.5 Framing Diosquinone as a Lead Scaffold

Overall, these data suggest that diosquinone is not an established EGFR inhibitor, but a structurally intriguing compound that should be explored for therapeutic applications as well. The compound has a high predicted affinity to achieve meaningful geometric complementarity with the EGFR binding site. Despite its non-classical interaction pattern, it does not follow the universal behaviour of typical TKI's, but it does highlight some specific structural characteristics that is the lack of an appropriate hydrogen bond donor or acceptor for hinge engagement that may be targeted for rational drug modification in further studies. This seems to be a definite challenge of its physicochemical properties, which could be optimized by medicinal chemistry. In this study, no enzymatic, cellular, or pharmacokinetic experiments have been conducted, nor have the results of these experiments been created. The computational results may not be extrapolated beyond the scope in which they were obtained and used. Diosquinone should be considered as an initial lead, rather than a confirmed hit.

5.6 Limitations

There are some drawbacks that should be recognized in the computational approach. The docking protocol used was a rigid model of the receptor that does not take into consideration conformational changes in the kinase domain of the EGFR following ligand binding. The use of molecular dynamics simulation or ensemble docking in future could provide a more realistic view of the binding behaviour [76]. SwissADME predictions are helpful as initial filters for pharmacokinetic parameters, but should not replace experimental ones in validated BBB assay systems, particularly metabolic stability, plasma protein binding, and permeability. Further, docking to the ATP binding site does not give any indication of selectivity against similar kinases, and selectivity profiling would be an integral part of any experimental downstream program. Lastly, scaffolds containing quinones may have intrinsic redox and cytotoxicity issues beyond prediction by ADME properties that would necessitate dedicated toxicological evaluation.

6. Conclusion

In the present study, an integrated computational workflow, namely molecular docking, residue-level interaction analysis and in silico ADME profiling, was used to assess a set of natural compounds as potential inhibitors of EGFR in glioblastoma multiforme. Three major conclusions were drawn. First, among all the compounds tested diosquinone had the highest predicted binding affinity, of -10.1 kcal/mol, which was higher than the reference inhibitor erlotinib with a predicted binding affinity of -7.2 kcal/mol. Second, residue-level interaction analysis showed that this affinity did not involve the typical hydrogen bonding interactions found in classical EGFR inhibitors, instead diosquinone stabilization in the ATP-binding pocket was mainly based on non-classical cation–π contacts. Third, pharmacokinetic profiling revealed that diosquinone had a limited predicted BBB permeability, with a TPSA of 121.27 Å², indicative of a significant barrier to CNS exposure in the GBM context. Together, these results demonstrate that docking affinity is not a reliable criterion for determining compound functionality as an EGFR inhibitor and that geometry and CNS pharmacokinetics are also

important, in addition to the energetics of binding. Diosquinone is rightly considered as a structurally interesting scaffold and not as a confirmed inhibitor and its progress will need to be validated in the laboratory and optimized through targeted physicochemical modification.

7. Future Scope

Results of this computational study can be used to guide the following experimental and analytical paths. To evaluate the stability of the diosquinone–EGFR complex under dynamic conditions, and to improve the predicted binding mode, molecular dynamics simulations and binding free-energy calculations should be conducted. In vitro EGFR kinase inhibition assays and studies in glioblastoma cell lines would confirm the predicted affinity and demonstrate biological activity. The empirical pharmacokinetic data obtained from BBB permeability assays using in vitro models validated for this purpose would validate or adjust the in-silico predictions. For subsequent virtual screening or synthesis campaigns, structural analogues of diosquinone that can be designed to contain hydrogen bond donors or acceptors that can be engaged in the hinge region and to decrease polar surface area for better CNS penetrance could be considered. Furthermore, the scaffold of diosquinone, which is based on a quinone structure, could be further explored as a potential electrophilic scaffold for the development of covalent or long-lived EGFR-targeting compounds. Future structural optimization studies may focus on modifications that would enhance interaction with nucleophilic residues in the kinase domain and also have CNS permeability and increase the stabilization of the hinge region.

Selectivity profiling of the kinases and some preliminary toxicological testing of any candidate analogues also would be required prior to preclinical advancement. In general, the computational workflow developed here could be expanded to search larger sets of natural products against the EGFR kinase domain, to systematically discover structurally diverse scaffolds relevant to GBM.

8. Social Impact

Despite some improvements in patient outcomes over the last few decades, GBM is still a major unmet clinical need, with a poor prognosis. The study of new molecules and candidate drugs in this disease, as a result, is directly relevant to a field in which there is a lack of therapeutic options. In addition to the specific results obtained in this study, the methodology used – open-access computational techniques and publicly available structural and pharmacokinetic data shows the possibility of doing early-stage drug candidate screening without the cost and resource demands of conventional experimental methods. This is important in the research environment, especially when lab facilities are limited, and can reduce the obstacles to hypothesis-driven research of potential therapeutic agents. The exploration of natural product-derived scaffolds is also in line with the overall strategy of searching for drugs from readily available and chemically diverse natural sources that could be useful for drug discovery in low-resource regions of the world.

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