



Network Pharmacology and Molecular Docking Insights into N-Mannich Base of Quinazolin-4(3H)-one Derivatives as Potential Multi-Target Agents against Glioblastoma and Inflammation

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ABSTRACT

Introduction: Glioblastoma (GBM) is the most aggressive primary brain tumor, and its growth is sustained by chronic inflammation and redundant signaling through EGFR, VEGFR and JAK-STAT pathways, which is why single-target therapy often fails. N-Mannich bases of quinazolin-4(3H)-one are attractive scaffolds for multi-target design. This study evaluated six such derivatives (3A–3F) as potential multi-target agents against glioblastoma and inflammation using computational methods.

Materials and Methods: Structures were drawn in ChemSketch and ChemDraw, and their physicochemical, ADME and toxicity profiles were predicted with SwissADME, pkCSM and ProTox-II. Targets were predicted with SwissTargetPrediction and overlapped with glioblastoma and inflammation genes from GeneCards. A protein–protein interaction network was built in STRING and analyzed in Cytoscape, and KEGG and GO enrichment were done with ShinyGO. Docking was performed in PyRx against EGFR (1M17), VEGFR (4ASD) and JAK1 (5E1E) and compared with gefitinib, sorafenib and upadacitinib.

Results and Discussion: All six compounds satisfied Lipinski's rule of five. ProTox-II predicted no hepatotoxicity, carcinogenicity, mutagenicity or cytotoxicity, with predicted LD₅₀ values of 255–2000 mg/kg. A total of 126 targets were shared between the compounds, glioblastoma and inflammation. The network had 51 nodes and 100 edges (PPI enrichment $P < 1.0 \times 10^{-16}$), with EGFR, JAK2 and ERBB2 as the top hub genes, and KEGG enrichment highlighted EGFR tyrosine kinase inhibitor resistance, JAK-STAT and PI3K-Akt signaling. Docking energies ranged from –7.3 to –8.3 kcal/mol (EGFR), –8.4 to –9.7 kcal/mol (VEGFR) and –7.7 to –9.0 kcal/mol (JAK1). Compound 3E scored best against all three targets (–8.3, –9.7 and –9.0 kcal/mol), stronger than gefitinib for EGFR and equal to upadacitinib for JAK1, but weaker than sorafenib for VEGFR.

Conclusion: Compound 3E was the most promising derivative, showing the strongest predicted binding to EGFR, VEGFR and JAK1 together with a favorable drug-likeness and safety profile. These results are computational only and need experimental validation.

Keywords: Glioblastoma; Quinazolin-4(3H)-one; N-Mannich base; Network pharmacology; Molecular docking; EGFR; JAK1.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the brain and is classified as a WHO grade IV neoplasm. It makes up roughly 16% of all primary brain and central nervous system tumors, with an age-adjusted incidence of about 3.2 per 100,000 people. The tumor originates from astrocytes, the star-shaped glial cells that support neurons, and its cells divide rapidly. Although GBM is located mainly in the cerebral hemispheres, it can also involve the brain stem, cerebellum and spinal cord. About 61% of primary gliomas are found in the cerebral lobes; the frontal lobe is the most frequent site (25%), ahead of the temporal (20%), parietal (13%) and occipital (3%) lobes^[2].

For a long time GBM was thought to arise only from glial cells. Current evidence instead suggests that several cell types with neural stem cell-like properties, ranging from stem cells to neurons and glia, can give rise to the tumor. Differences in tumor phenotype seem to depend mainly on altered molecular signaling pathways rather than on the cell of origin (Figure 1)^[1].

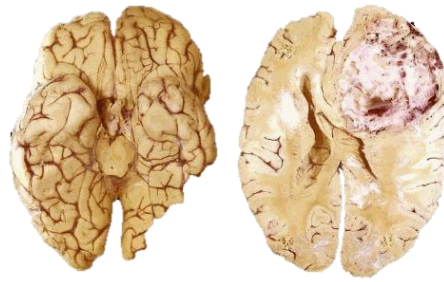


Figure 1: Image of Normal and Glioblastoma Brain

CAUSES AND RISK FACTORS

The cause of GBM and other brain tumors remains largely unknown, but a few risk factors have been identified^[2]:

Ionizing radiation: High-dose ionizing radiation is the only environmental exposure conclusively linked to the disease.

Genetic predisposition: Heritable factors explain about 5–10% of cases. Rare inherited syndromes, including neurofibromatosis types 1 and 2 and tuberous sclerosis, are associated with a raised incidence.

Previous cancer therapy: Survivors of acute lymphoblastic leukemia (ALL) seem to be at greater risk, which may be due to earlier cranial radiotherapy or certain chemotherapy drugs.

SIGNS AND SYMPTOMS

GBM symptoms generally progress quickly, often within 3 to 6 months of onset, and can be grouped according to three underlying mechanisms^[2]:

1. Local Tissue Damage (Focal Deficits)

As the tumor destroys adjacent brain tissue, focal neurological deficits appear, and their nature depends on the tumor's location:

Cognitive and personality changes: reported in roughly 20–40% of patients whose tumor lies in the frontal lobe.

Visual and auditory disturbances: arise when the temporal lobe is affected.

Gait and balance problems: bulky tumors with a significant mass effect may lead to an unsteady gait and urinary incontinence.

2. Raised Intracranial Pressure

As the tumor grows and surrounding edema develops, the pressure within the skull rises, producing:

Progressive headache: present in about 30–50% of patients; it is typically unilateral and becomes worse over time.

Vomiting: frequently occurs together with a severe headache.

Papilledema: swelling of the optic disc, now seen less frequently because tumors are diagnosed earlier.

3. Seizures

Epileptic episodes: affect around 20–40% of patients; they may be simple partial, complex partial or generalized and usually begin focally^[2].

INFLAMMATION

Inflammation is the protective response of living, vascularized tissue to harm from any cause. It serves to neutralize or isolate the injurious agent and to remove necrotic cells and debris, so that tissue repair can start^[3].

CAUSES

A wide range of injurious agents can initiate inflammation, including:

- Microbial infection by bacteria, viruses (and their toxins), fungi or parasites.
- Immune-mediated injury, for instance cell-mediated reactions and antigen–antibody interactions.
- Physical insults such as extreme heat or cold, radiation and mechanical trauma.

- Chemical injury from organic or inorganic toxic substances.
- Foreign bodies and other inert material.

SIGNS OF INFLAMMATION

In the first century A.D., the Roman author Celsus identified redness (rubor), swelling (tumor), heat (calor) and pain (dolor) as the four cardinal signs of inflammation^[3].

Virchow later added impaired function (functio laesa) as a fifth sign^[3]. The word “inflammation” derives from a Latin term meaning to set on fire, which reflects early bedside observation; burning is now known to be only one of several features of the response.

TYPES OF INFLAMMATION

Inflammation is classified as acute or chronic according to how long the response lasts and how the host reacts.

A. Acute inflammation sets in rapidly and is the body's first line of response; it generally lasts less than two weeks and commonly ends in healing.

Typical features include:

- Leakage of fluid and plasma proteins into the injured tissue
- Platelet activation inside blood vessels, together with
- accumulation of polymorphonuclear neutrophils as the dominant inflammatory cells.

When the acute reaction is especially severe, it is termed fulminant acute inflammation.

B. Chronic inflammation is longer-lasting and may follow a delay. It arises when the cause of the acute response is not removed, or when the stimulus triggers a chronic reaction from the start. Chronic active inflammation is a subtype in which acute flare-ups interrupt the chronic course.

Its hallmark is the presence of chronic inflammatory cells (lymphocytes, plasma cells and macrophages), together with granulation tissue formation and, in certain diseases, granulomas^[3].

GLIOBLASTOMA-ASSOCIATED INFLAMMATION

Chronic inflammation is a hallmark of glioblastoma and plays an important role in tumor growth and progression. The tumor microenvironment contains many tumor-associated macrophages and microglia, which shift toward an immunosuppressive, pro-tumor (M2-like) state. These cells release cytokines and mediators such as IL-6, IL-1 β , TNF- α , TGF- β , VEGF and prostaglandin E2 (via COX-2). These signals activate the NF- κ B and STAT3 pathways, which promote tumor cell proliferation, survival, invasion and angiogenesis. Hypoxia and necrosis in the tumor core further increase inflammatory signaling, and the resulting inflammation also suppresses T-cell responses and contributes to resistance to temozolomide and radiotherapy. Compounds that combine antiproliferative and anti-inflammatory activity are therefore of interest for glioblastoma, which supports evaluating the synthesized quinazolin-4(3H)-one N-Mannich bases.

QUINAZOLINONES AND MANNICH BASES

Quinazolin-4(3H)-one is a nitrogen heterocycle whose chemistry and structure–activity relationships have been studied widely^[4]. Quinazolinone derivatives show a broad range of activities, including anticancer and anti-inflammatory effects^[5–7], and some have been designed as EGFR inhibitors for antitumor treatment^[8].

Mannich bases, formed by aminoalkylation of an active hydrogen, are useful in medicinal chemistry and drug design^[9]. Because glioblastoma depends on EGFR, VEGFR and JAK-STAT signaling together with inflammation, this study used ADME and toxicity prediction, network pharmacology and molecular docking to evaluate six N-Mannich bases of quinazolin-4(3H)-one (3A–3F) as potential multi-target agents.

MATERIALS AND METHODS

Molecular Sketching and SMILES Generation

The structural architectures of the newly designed target chemical entities were initially rendered in two dimensions (2D) utilizing the ChemSketch software suite (Version 12.0). Following structural validation, the chemical configurations were programmatically converted into their respective Simplified Molecular-Input Line-Entry System (SMILES) strings. These canonical line notations served as the standardized computational input format required for subsequent web-based predictive algorithms and molecular descriptors.

In Silico ADME and Physicochemical Profiling

The primary evaluation of the biophysical, pharmacokinetic, and drug-like properties of the target molecules was performed using the SwissADME web server (swissadme.ch), hosted by the Swiss Institute of Bioinformatics (SIB)^[10]. The SMILES notations for each compound were submitted to the server to compute critical physicochemical parameters, including molecular weight (MW), lipophilicity Log Po/w, topological polar surface area (TPSA), and hydrogen bond donor/acceptor counts.

Computational Toxicity and Lethal Dose Assessment

To establish the preliminary safety profile and hazard potential of the synthesized compounds, comprehensive in silico toxicity screenings were performed using two complementary in silico platforms: ProTox-II (charite.de) and pkCSM (uq.edu.au).

The ProTox-II server^[11] was utilized to determine the acute oral toxicity by calculating the predicted median lethal dose (LD₅₀) values expressed in milligrams per kilogram body weight (mg/kg). Based on these LD₅₀ projections, the compounds were stratified into specific Globally Harmonized System (GHS) toxicity classes (Classes 1–6). The platform also mapped toxicological endpoints, including organ toxicity (hepatotoxicity) and toxicological pathways (nuclear receptor signaling and stress response pathways). Concurrently, the pkCSM web server was employed as a secondary validation tool to predict additional endpoints, such as Ames mutagenicity, maximum tolerated human dose, skin sensitization potential, and chronic toxicity endpoints, ensuring a robust toxicological prediction profile.

TARGET PREDICTION BY NETWORK PHARMACOLOGY APPROACH

Initially, the targets for the synthesized compounds were predicted by using the Swiss target prediction on the Swiss ADME web server^[12]. The most prominent and highly active targets from the Swiss target prediction were chosen for further molecular docking and biological studies.

Construction of Target Protein-Protein Interaction (PPI) network

Protein-protein interaction (PPI) analysis was done using the protein targets using STRING 3.0 database (<https://string-db.org/>). A minimum interaction network was chosen for further analysis using Cytoscape. For the PPI network, initially, common genes associated with the predicted targets using Swiss target prediction and the disease targets using Venn plot (<https://bioinformatics.psb.ugent.be/webtools/Venn/>)

Enrichment analysis and KEGG pathway

The gene enrichment analysis and KEGG pathway of the title compounds were obtained using Shinygo 0.77 webserver (<http://bioinformatics.sdstate.edu/ge/>) by employing the common genes obtained from the Venn plot. The top pathways are selected and the top most active target against the synthesized compounds was identified.

Network Visualization

The networks were constructed by utilizing the network visualization software Cytoscape^[13]. Moreover, some networks were directly exported from the in situ web-based platform like the STRING database. The predictor targets and network against cancer and inflammation were obtained in simple hierarchical networks. Thus, based on the most prominent targets and common pathways obtained from the Swiss target prediction and Network pharmacology approach, the targets for the molecular docking studies and in vitro techniques were selected.

MOLECULAR DOCKING

Ligand Preparation

A list of 6 synthetic compounds designed was given in the scheme. The structures of these compounds were sketched using ChemDraw Ultra 12.0 (www.cambridgesoft.com). The 3D structures were exported for energy minimization and geometry correction^[14]. The outputs of the structures were saved (pdb) format, which were used for further in silico studies.

Protein preparation and validation

Crystal three-dimensional structures of selected targets based on network pharmacology were downloaded from RCSB Protein Data Bank (<http://www.rcsb.org>) in the pdh format. The stereochemical properties of each target protein were assessed based on the information obtained from the PDB X-Ray structure Validation Report for each PDB structure. The resolution and R-values showed the goodness of the protein model being used. Proteins were energy minimized, water molecules and cocrystallized ligands were removed, polar hydrogen and Kollman charges were added. Finally, 3D structures of the target proteins were saved in pdbqt format and used for docking studies.

Molecular docking and visualization

Molecular docking was performed by using PyRx software. The docking parameters selected were 20 docking runs and a population size of 150. The binding energy of the protein coordinate was evaluated by a three-dimensional grid box of 60x60x60 (Number of grid points in xyz) was created with a spacing of 0.375 Å^[15]. Biovia Discovery Studio version 21.1.0. (<https://www.3dsbiovia.com/>) was used to visualize the docking results. The docking energies of the synthesized compounds were compared with the commercially available anti-cancer drugs and co-crystallized ligands for the validation

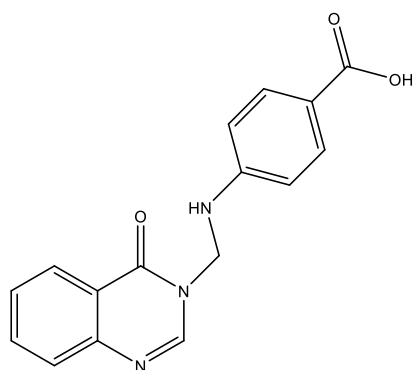
of results. The docking energy for a given protein-ligand pair comprised of the intermolecular interaction energies including internal steric energy, hydrogen bond interaction energy. Van der Waals force and columbic electrostatic energy of the compounds. The lowest binding energy of the protein-ligand complex has been considered to be the best.

RESULTS AND DISCUSSION

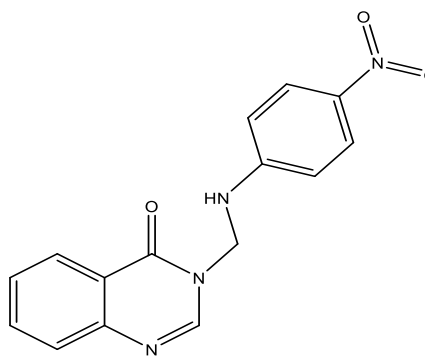
Designed compounds

The six derivatives share the Quinazoline-4(3H)-ONE core and differ only at N-3 (Figure 2).

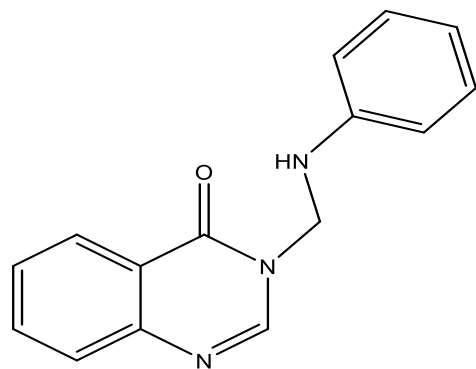
3A



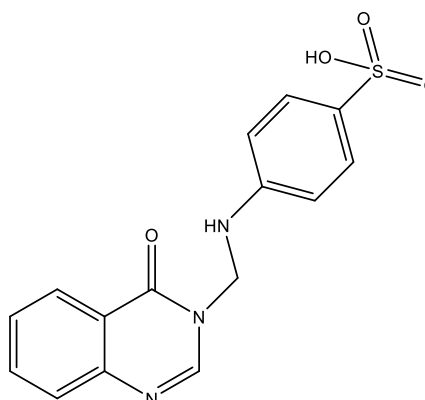
3B



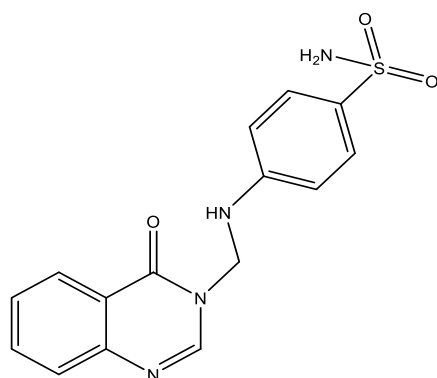
3C



3D



3E



3F

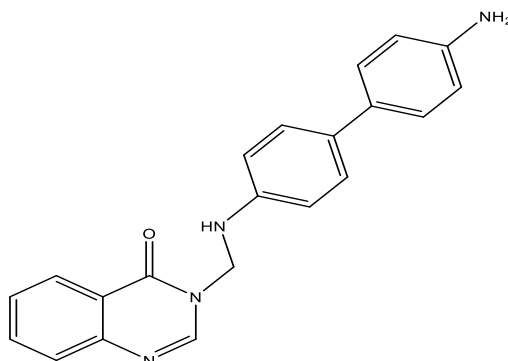


Figure 2: Structures of the designed derivatives 3A-3F

LIPINSKI'S RULE OF FIVE

Lipinski's Rule of Five was used to evaluate the drug-likeness of the synthesized compounds (Table 1).

Compound ID	Molecular formula	Molecular weight	H-Bond donors	H-Bond acceptors	Log-p	Molar refractivity	No. of Criteria follow
Rule		<500 g/mol	<5	<10	<5	<150	Min-3
3A	C ₁₆ H ₁₃ N ₃ O ₃	295.29	2	5	2.24	83.05	5
3B	C ₁₅ H ₁₂ N ₄ O ₃	296.28	1	5	2.97	84.91	5
3C	C ₁₅ H ₁₃ N ₃ O	251.29	1	3	2.54	76.09	5
3D	C ₁₅ H ₁₃ N ₃ O ₄ S	331.35	2	6	2.87	85.95	5
3E	C ₂₁ H ₁₈ N ₄ O	342.39	2	4	3.37	105.93	5
3F	C ₁₅ H ₁₄ N ₄ O ₃ S	330.36	2	6	2.97	87.09	5

Table 1: Physicochemical properties of the synthesized compounds by using SWISSADME Webserver

ADME PROPERTIES OF THE SYNTHESIZED COMPOUNDS

To evaluate the drug-likeness of the synthesized compounds, their Absorption, Distribution, Metabolism, and Excretion (ADME) parameters were predicted using the online pkCSM webserver. The detailed results are consolidated in Table 2.

1. Absorption

Caco-2 Permeability : Caco-2 cell monolayers are used to model absorption across the intestinal wall. In pkCSM, a compound is predicted to be highly permeable when its log P_{app} exceeds 0.90 (P_{app} above 8×10^{-6} cm/s)^[16]. The synthesized compounds generally showed favorable permeability.

Human Intestinal Absorption (HIA): HIA is the predicted proportion of a compound absorbed from the human small intestine; values below 30% are taken to indicate poor absorption^[16]. Most of the compounds showed high predicted absorption, suggesting good oral bioavailability.

2. Distribution

Steady-State Volume of Distribution (logVDss): VDss is the theoretical volume in which a dose would need to be distributed to match its plasma concentration at steady state. In pkCSM, logVDss below -0.15 is considered low and above 0.45 high^[16]. The compounds fell in the low range.

Fraction Unbound: the proportion of the compound not bound to plasma proteins and therefore free to cross cell membranes^[16].

Blood-Brain Barrier (BBB) & CNS Permeability: Predicted logBB values above 0.3 indicate ready brain penetration, whereas values below -1.0 indicate poor brain distribution; CNS permeability (log P_s) gives a further measure of brain access, relevant to both central efficacy and neurotoxicity^[16].

3. Metabolism

Cytochrome P450 (CYP) Models: Hepatic CYP enzymes drive much of drug metabolism. pkCSM predicts whether a compound is a substrate or inhibitor of the main isoforms (here CYP2D6, CYP3A4 and CYP1A2)^[16].

4. Excretion

Total Clearance (logCL_{tot}) : Expressed as log(mL/min/kg), this value reflects combined hepatic and renal clearance and informs dosing rate^[16].

Renal OCT2 Substrate: OCT2 is a key renal uptake transporter; whether a compound is a substrate helps flag possible drug-drug interactions and altered renal clearance^[16].

Property	Parameters	3A	3B	3C	3D	3E	3F
ABSORPTION	CaCO ₂ permeability (log Papp in 10cm/s)	0.996	0.795	1.303	0.044	0.925	0.008
	HIA (%)	98.081	91.032	93.965	57.765	92.068	78.835
DISTRIBUTION	VDss (log L/kg)	-1.0304	-0.285	-0.177	0.774	-0.276	-0.341
	Fraction unbound	0.125	0.058	0.142	0.139	0.120	0.132
	BBB Permeation (log BB)	-0.414	-0.338	-0.381	-0.824	-0.202	-0.764
	CNS Permeation (log Ps)	-2.438	-2.241	-2.314	-2.729	-2.125	-2.813
METABOLISM	CYP2D6	NO	NO	YES	NO	YES	NO
	CYP3A4	YES	NO	YES	YES	YES	YES
	CYP1A2	YES	NO	YES	NO	YES	NO
EXCRETION	Total Clearance (log ml/min/kg)	0.51	0.376	0.561	0.396	0.341	0.745
	Renal OCT2 Substrate	NO	NO	NO	NO	NO	NO

Table 2: ADME properties of the synthesized compounds by using pkCSM webserver

Note: Caco: (Human epithelial colorectal adenocarcinoma cells) Permeability; PGP-P-Glycoprotein; HIA-Human Intestinal Absorption; VDss- Volume of Distribution; BBB-Blood brain barrier; CYP-Cytochrome P450 enzymes; Renal Organic Cation Transporter.

TOXICITY ASSESSMENT OF THE SYNTHESIZED COMPOUNDS

The majority of the small organic molecules failed to convert into drug substances due to their toxic effects. Computational toxicity estimations are not only faster than finding dangerous or toxicity levels using animals, but they can also help to reduce the number of animal tests. Predicting the fatal dose of a possible chemical is critical. The lethal dosage values are a common measure of acute toxicity that can be used to compare the toxicity profile of various compounds. The LD₅₀ is the amount of a substance that, when given to a group of test microorganisms or animals, results in a 50% inhibitory or survival rate. The toxicity class was also predicted. The toxicity class 1, 2 and 3 were found to produce potential toxic effects. The synthesized Mannich base ligands were found to be of class 4 and 5. In order to identify the various toxicity profile of the synthesized compounds, the hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity and immunogenicity was determined by using the PROTOX-II webserver (Table 3). All of the synthesized compounds are found to be non-cytotoxic, non-cardiotoxic, non-hepatotoxic and non-carcinogenic agents. All of the synthesized compounds were found to be non-cytotoxic in nature i.e., all the compounds are compatible with normal cells.

COMPOUND ID	3A	3B	3C	3D	3E	3F
Hepatotoxicity	-	-	-	-	-	-
carcinogenicity	-	-	-	-	-	-
Immunogenicity	-	-	+	-	-	-
Mutagenicity	-	-	-	-	-	-
cytotoxicity	-	-	-	-	-	-
Predicted LD ₅₀	500	500	255	1000	500	2000
Toxicity class	5	5	3	5	5	5

Table 3: Toxicity assessment of the synthesized compounds by using PROTOX-II webserver

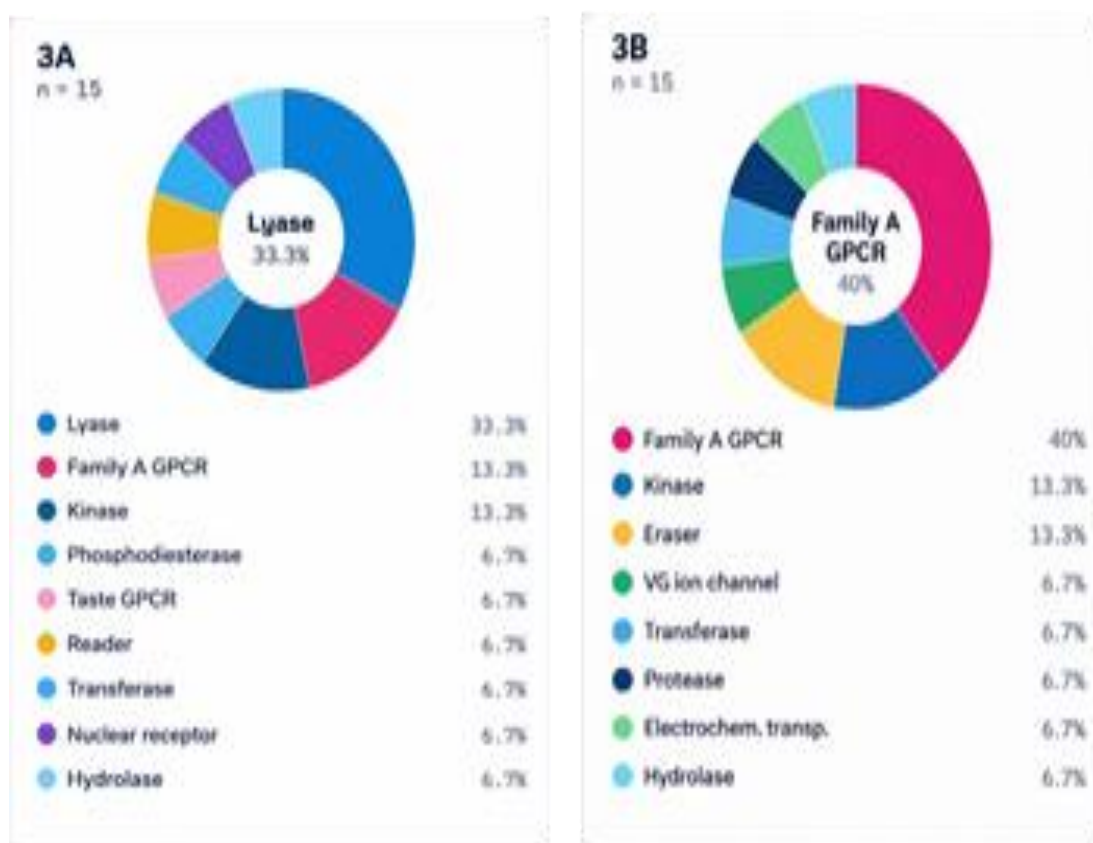
Note: - toxicity not predicted ; + toxicity predicted ,LD: Lethal dose (mg/kg); Class 1: Fatal if swallowed ($LD_{50} \leq 5$); Class 2: Fatal if swallowed ($5 < LD_{50} < 50$), Class 3: Toxic if swallowed ($50 < LD_{50} \leq 300$); Class 4: Harmful if swallowed ($300 < LD_{50} \leq 2000$); Class 5: May be harmful if swallowed ($2000 < LD_{50} \leq 5000$)

NETWORK PHARMACOLOGY

POTENTIAL TARGETS OF SYNTHESIZED N-MANNICH BASE OF QUINAZOLINE-4(3H)-ONE DERIVATIVES

The *in silico* analysis of the synthesized N-Mannich base of quinazoline-4(3H)-one derivatives evaluated a total of 6 synthesized compounds for their therapeutic potential. These compounds were systematically screened for drug-likeness and oral bioavailability using Lipinski's Rule of Five (Ro5). Compounds satisfying these essential pharmacokinetic criteria, particularly those optimized for blood-brain barrier (BBB) permeability, were selected for downstream target prediction using the Swiss Target Prediction database (<http://www.swisstargetprediction.ch>). Notably, derivatives featuring electron-withdrawing groups and specific primary amino networks met the strict criteria for drug-likeness and demonstrated highly promising bioavailability profiles. These active derivatives were submitted to Swiss Target Prediction to map their potential human protein targets. A detailed overview of the predicted targets and compound-receptor interactions is illustrated in Figure 3.

Using the GeneCards database, a comprehensive dataset of genes was identified to be critically associated with glioblastoma multiforme (GBM) and chronic inflammatory pathways based on their relevance scores. This dense genetic dataset reflects the multifactorial pathogenesis of GBM and its interconnected inflammatory microenvironment, which encompasses unconstrained cell proliferation, evasion of apoptosis, microglial activation, neuroinflammation, and resistance to standard therapeutics. The key genes identified included CDK5, EGFR, COX-2, NLRP3, TNF, and IL6, all of which play central, driving roles in tumor progression, cell cycle deregulation, and sustained pro-inflammatory signaling cascades.



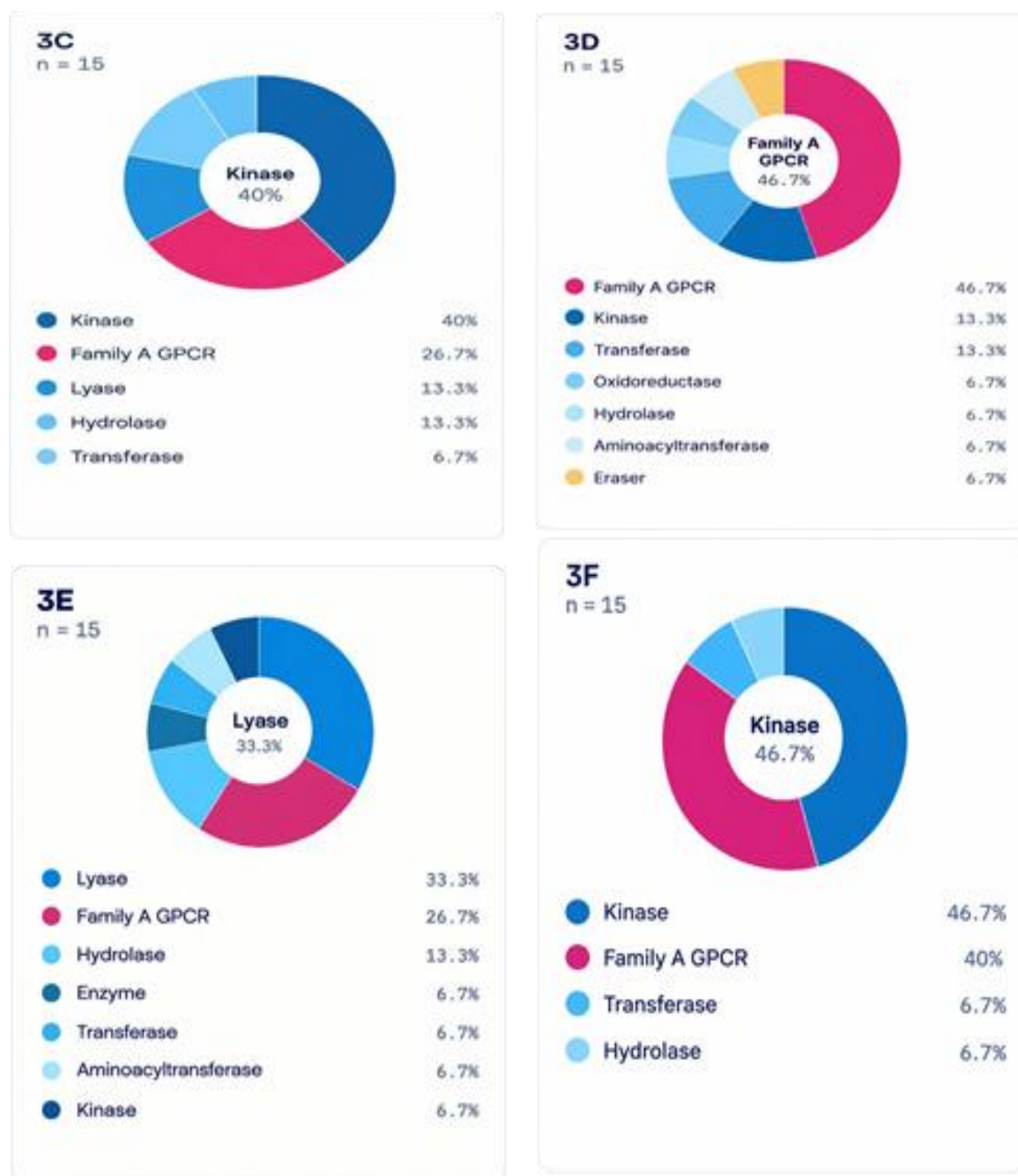


Figure 3: Predicted molecular targets of synthesized compounds

IDENTIFICATION OF SHARED THERAPEUTIC TARGETS

To identify the potential therapeutic targets of N-Mannich base quinazoline-4(3H)-one derivatives in the context of glioblastoma multiforme (GBM) and inflammation, we conducted a target overlap analysis using Venn diagram visualization. A total of 126 overlapping targets were identified as common among the quinazoline-4(3H)-one derivatives, the GBM dataset, and inflammatory disease profiles. This suggests their potential relevance in mediating the therapeutic effects of these novel derivatives by simultaneously targeting glioblastoma and inflammatory cascades.

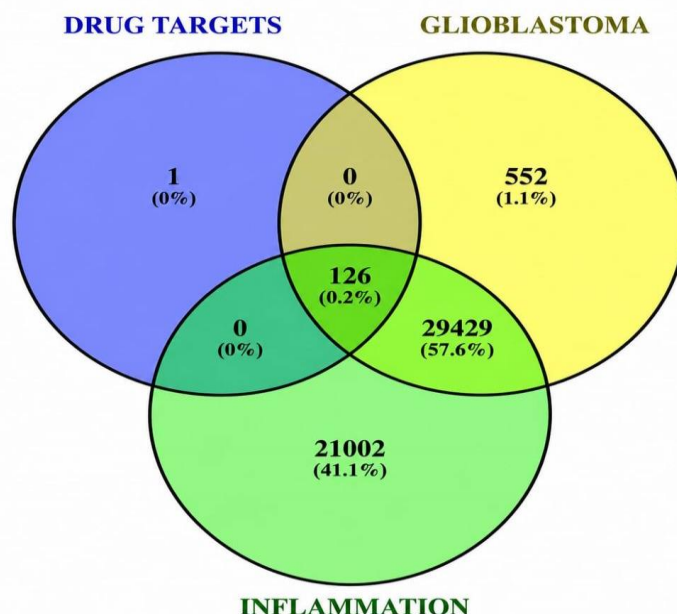


Figure 4: Target Venn diagram of synthesized compounds, Glioblastoma and Inflammation

CONSTRUCTION OF PPI NETWORK:

To explore the mechanistic basis of the synthesized N-Mannich base quinazoline-4(3H)-one derivatives in mitigating glioblastoma multiforme (GBM) progression and associated inflammation, a protein-protein interaction (PPI) network analysis was performed using the STRING database. A total of 126 shared human protein targets, identified from the intersection of the derivative-target interactome and the dual-disease datasets. These proteins were processed using the STRING platform to construct a functional connectivity network, as illustrated in Figure 5

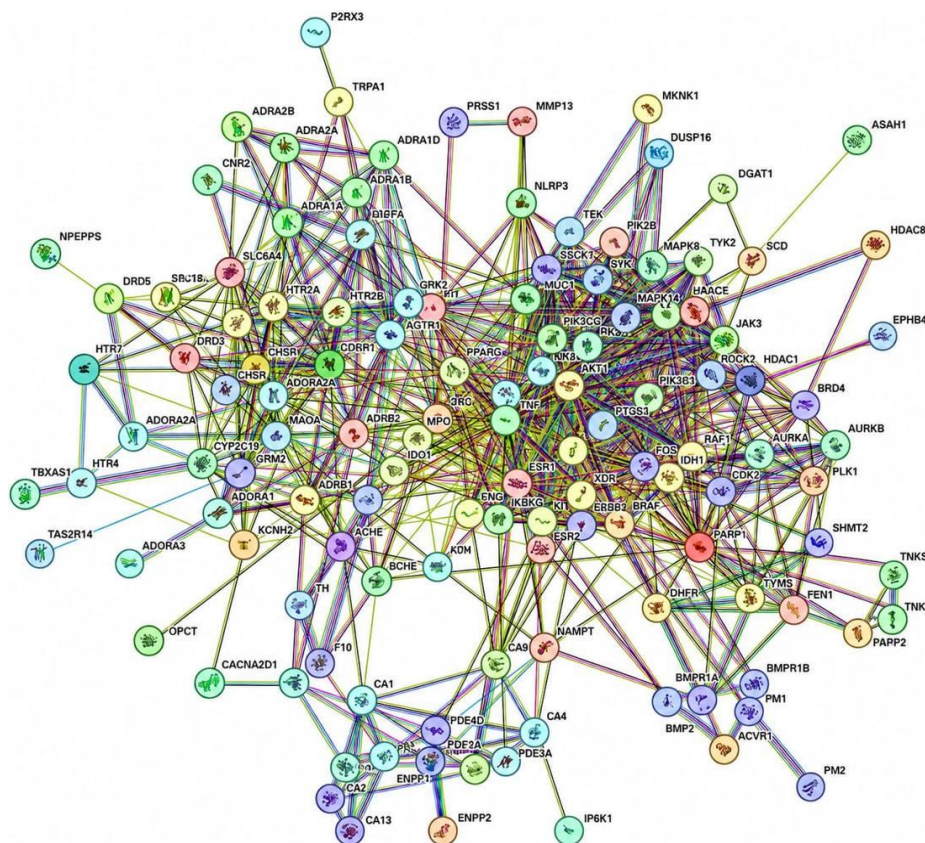


Figure 5: Protein - Protein Interaction

NETWORK STATISTICS (FROM STRING)

- **Number of Nodes (51):** Out of the 126 overlapping genes found in the Venn diagram, 51 core proteins were confirmed to physically interact and work directly with one another inside the human body.
- **Number of Edges (100):** The network contains exactly 100 direct communication lines or interaction paths connecting these 51 target proteins together.
- **Average Node Degree (3.92):** On average, each protein is directly linked to approximately 4 other proteins in the network, ensuring that modulating a single target initiates a robust cascading effect to disrupt the pathological framework.
- **Average Local Clustering Coefficient (0.443):** On a scale from 0 to 1, a **score of 0.443** is highly significant, confirming that the target proteins form close-knit, highly organized functional modules to control disease pathways.
- **Expected Number of Edges (42):** If a randomized baseline group of 51 unrelated proteins were evaluated, they would share only 42 connections by pure coincidence, whereas the active interactome contains 100 edges (more than double), demonstrating highly specialized biological selection.
- **PPI Enrichment P-value ($< 1.0e-16$):** This value is practically zero, providing definitive mathematical proof that these interactions did not occur by random chance and confirming that the synthesized derivatives target a coordinated, unified disease network.

These values indicate that the network possesses significantly more interactions than expected by chance, suggesting strong functional interconnectivity among the predicted targets of N-Mannich base quinazoline-4(3H)-one derivatives

To identify key molecular targets involved in the therapeutic mechanism of N-Mannich base quinazoline-4(3H)-one derivatives, topological analysis of the protein-protein interaction (PPI) network was performed. Degree centrality was used as a key parameter to rank nodes (genes/proteins) based on the number of direct interactions with other nodes, identifying potential hub genes critical to network stability and biological function. The top 10 hub genes are shown in Figure 6 and ranked in Figure 7 and Table 4.

Figure 6: Top 10 hub genes (color transitions from yello to orange indicates higher degree Values)

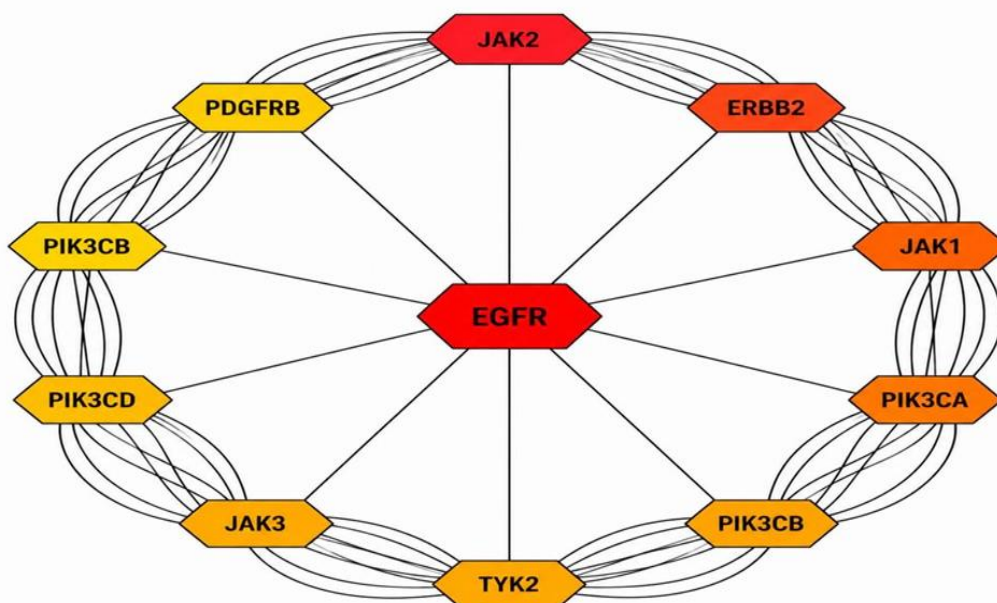
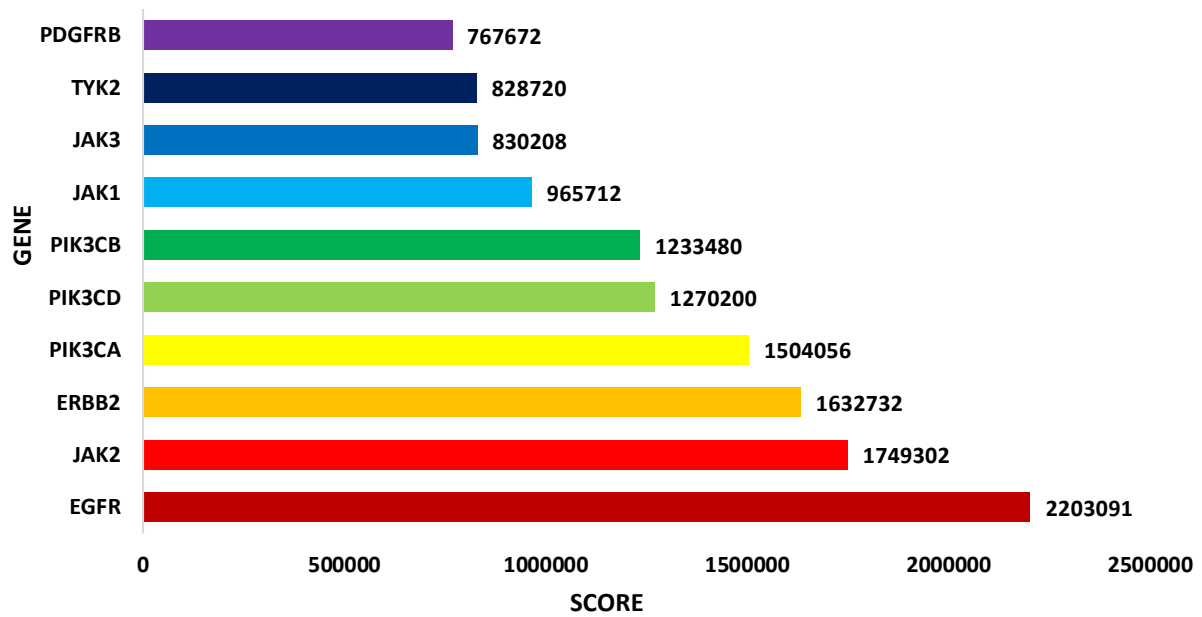


Figure 7: Ranking of Top 10 hub Genes Based on Gene Score

GENE SCORE DISTRIBUTION



Rank	Gene Symbol	Full Name	Biological Role
1	EGFR	Epidermal Growth Factor Receptor	Cell proliferation, wound healing, and inflammatory response
2	JAK2	Janus Kinase2	Regulates immunity, blood cell production, and inflammatory cytokine paths
3	ERBB2	ErbB-2 Receptor Tyrosine Kinase 2	Drives cell survival, expansion, and rapid cellular multiplication
4	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate Catalytic Subunit Alpha	Primary master engine for metabolic cell growth and survival signaling
5	PIK3CD	Phosphatidylinositol-4,5-Bisphosphate Catalytic Subunit Delta	Controls the development, signaling, and migration of adaptive immune cells
6	PIK3CB	Phosphatidylinositol-4,5-Bisphosphate Catalytic Subunit Beta	Governs cellular growth, metabolism, and specific platelet activations
7	JAK1	Janus Kinase1	Essential for cellular responses to type I/II interferons and inflammation
8	JAK3	Janus Kinase3	Vital step for white blood cell homeostatic development and activation
9	TYK2	Tyrosine Kinase2	Mediates immune responses against viral or bacterial infections
10	PDGFRB	Platelet-Derived Growth Factor Receptor Beta	Growth Factor Receptor BetaHandles structural blood vessel formation, tissue remodeling, and cell migration

Table 4: Top 10 hub genes

KEGG PATHWAY ANALYSIS

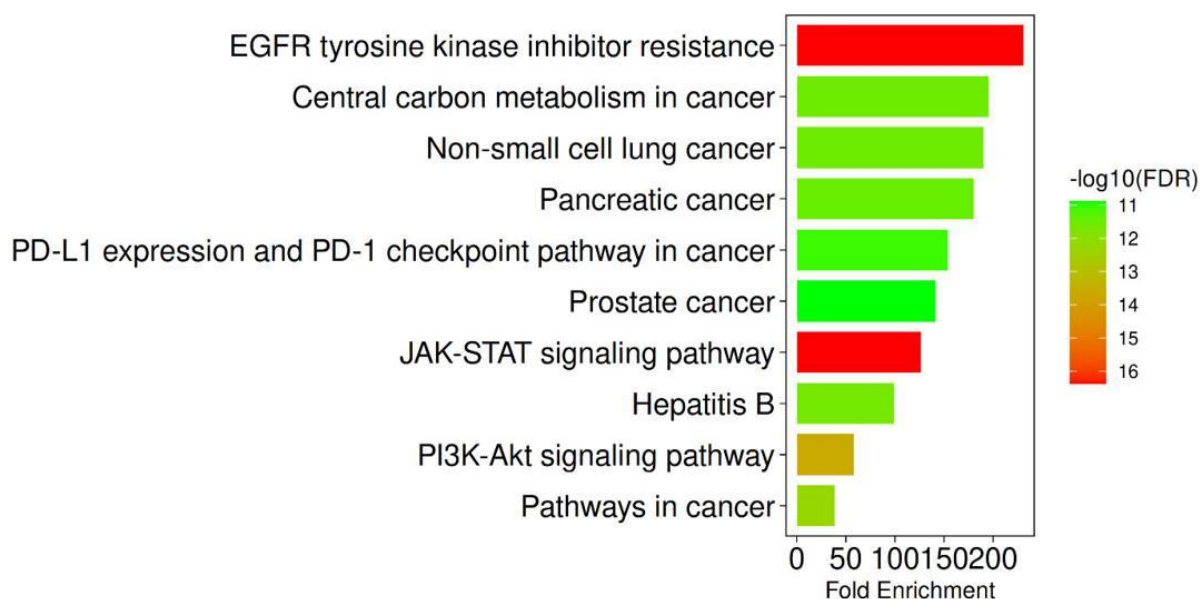


Figure 8: KEGG pathway enrichment analysis

To elucidate the potential biological processes and molecular mechanisms modulated by the synthesized N-Mannich base of quinazoline-4(3H)-one derivatives, a KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was performed. The results demonstrated that the target genes were significantly enriched in multiple key oncogenic and regulatory networks, including EGFR tyrosine kinase inhibitor resistance, Central carbon metabolism in cancer, Non-small cell lung cancer, Pancreatic cancer, PD-L1 expression and PD-1 checkpoint pathway in cancer, Prostate cancer, JAK-STAT signaling pathway, Hepatitis B, PI3K-Akt signaling pathway, and generalized Pathways in cancer, as illustrated in **Figure 8**.

These enriched pathways highlight the multi-target and multi-pathway nature of the functionalized quinazoline-4(3H)-one framework. The synthesized compounds may play a crucial role in modulating core cellular processes such as aberrant cell survival, immune checkpoint signaling, and chronic inflammation—all of which are vital therapeutic markers for tackling inflammation-associated glioblastoma progression.

NETWORK GRAPH OF OVERLAPPING TARGET PATHWAYS

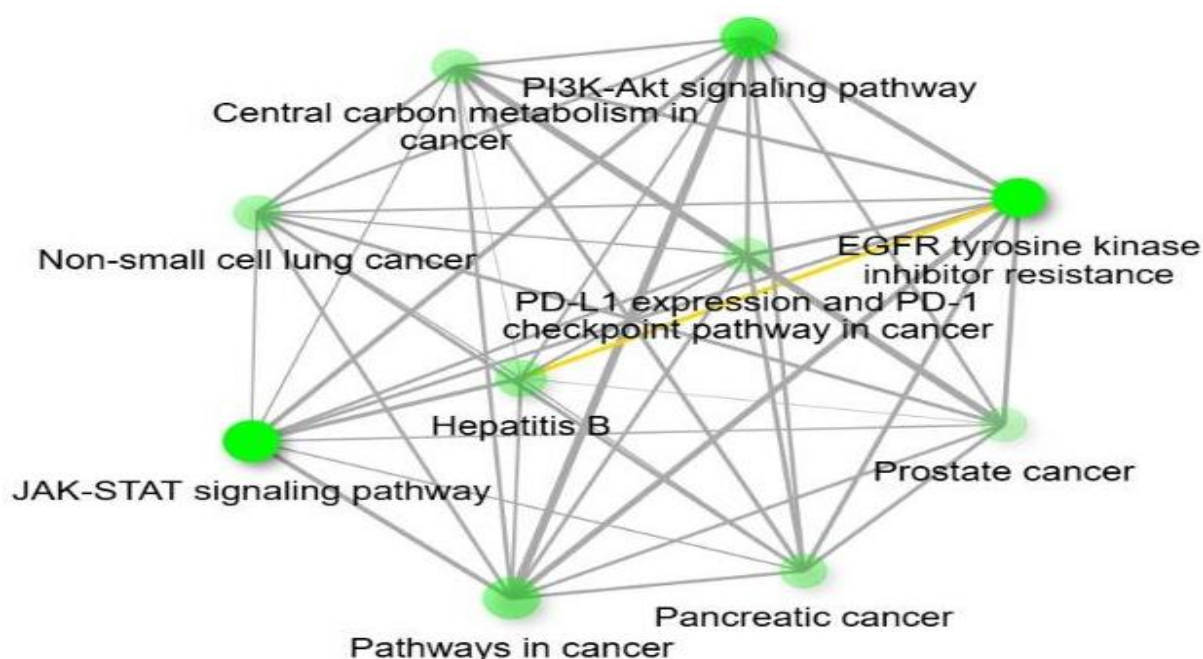


Figure 9: KEGG pathway enrichment network graph of overlapping target genes.

- EGFR tyrosine kinase inhibitor resistance pathway and the JAK-STAT signaling pathway emerge as primary core hubs. These primary therapeutic nodes display extensive structural interactions with both the PI3K-Akt signaling pathway and broader generalized Pathways in cancer (Figure 9).
- Crucially, the highlighted yellow edge explicitly maps a direct functional link between EGFR resistance mechanisms and the PD-L1 expression and PD-1 checkpoint pathway in cancer, demonstrating a bridge between growth factor hyperactivation and tumor immune evasion.
- This dense web of overlapping target genes proves that glioblastoma cells rely on highly redundant and shared biological machinery to sustain rapid growth, migrate, and resist cell death.
- The overall network architecture visually demonstrates that mono-targeted therapies inevitably trigger resistance due to these easy bypass loops, thereby strongly validating the multi-target design of the synthesized compounds which simultaneously shut down these overlapping circuits.

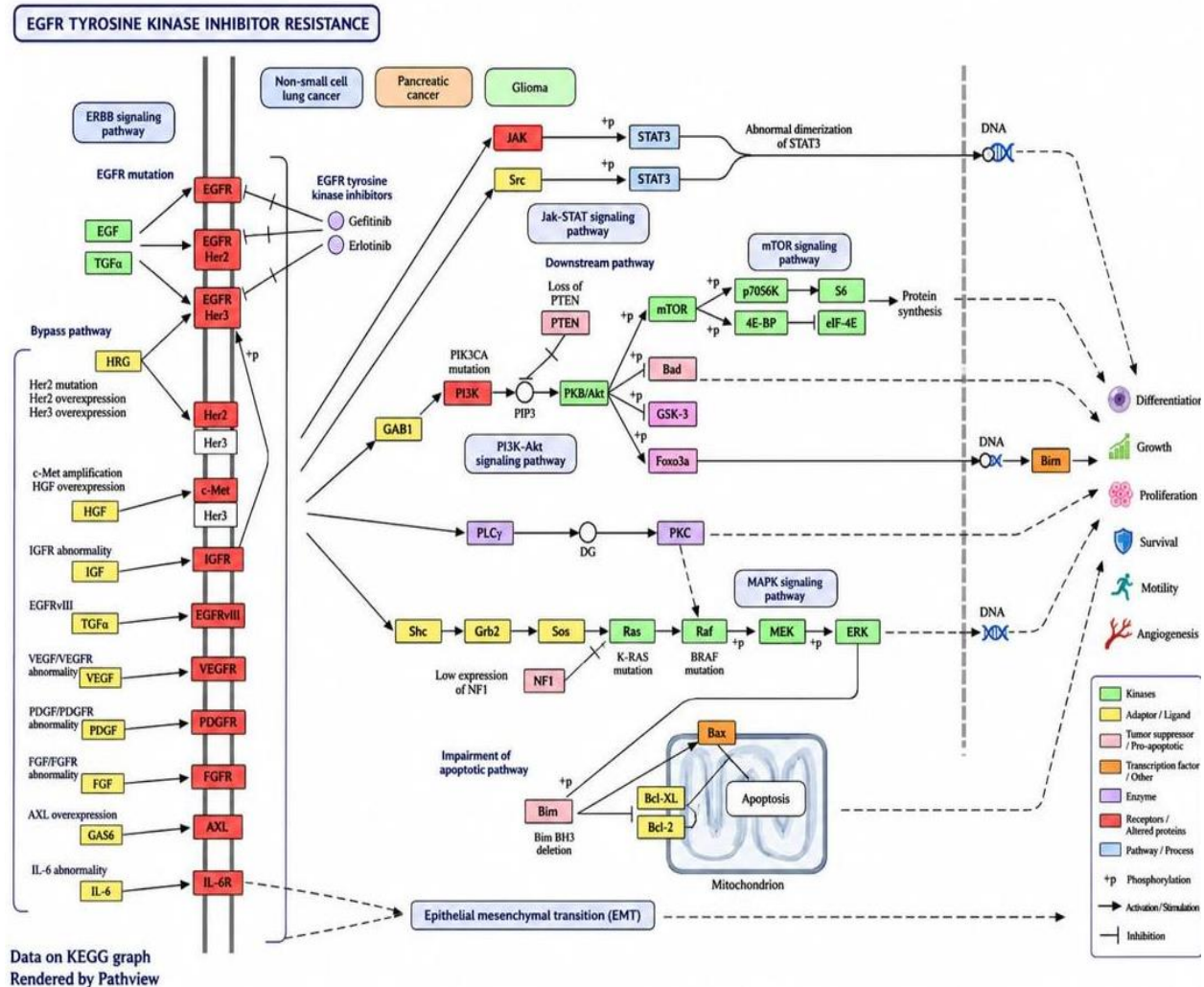


Figure 10: Schematic diagram of EGFR resistance and signaling pathways in Glioma

The KEGG pathway demonstrates how over-activated or mutated membrane EGFR initiates an interconnected signaling network to drive rapid glioblastoma progression. This hyperactive receptor undergoes functional crosstalk to activate downstream JAK1 and JAK2, forcing oncogenic STAT3 into the DNA nucleus to promote cell proliferation, migration, and survival. Concurrently, these upstream signals branch into the PI3K-Akt and MAPK pathways, while inflammatory IL-6/IL-6R cytokine signals feed into the same JAK network to create drug-resistance bypass loops. Furthermore, the pathway cross-talks with VEGFR to trigger tumor angiogenesis (Figure 10).

GENE ONTOLOGY (GO) ENRICHMENT ANALYSIS

Gene Ontology (GO) enrichment analysis of the N-Mannich base of quinazoline-4(3H)-one nucleus derivatives revealed significant involvement in key biological functions relevant to Inflammation and Glioblastoma.

Biological Process (BP)

- Regulation of phosphatidylinositol 3-kinase signaling
- MAPK cascade and protein phosphorylation **Figure 11**

These processes suggest a role in suppressing tumor proliferation, reducing neuro-inflammation, and controlling abnormal cell survival, crucial in glioblastoma and inflammation management.

Cellular Component (CC):

- Phosphatidylinositol 3-kinase complex, focal adhesion, cell-substrate junction, and membrane protein complex **Figure 13**

These are active roles in signal transduction, cell invasion pathways, and cellular integrity in glioblastoma cells.

Molecular Function (MF):

- Transmembrane receptor protein tyrosine kinase activity, protein kinase activity, and ATP binding **Figure 12**

These functions are critical for modulating downstream oncogenic pathways, kinase activity, and inflammatory responses key factors in glioblastoma and inflammation pathology.

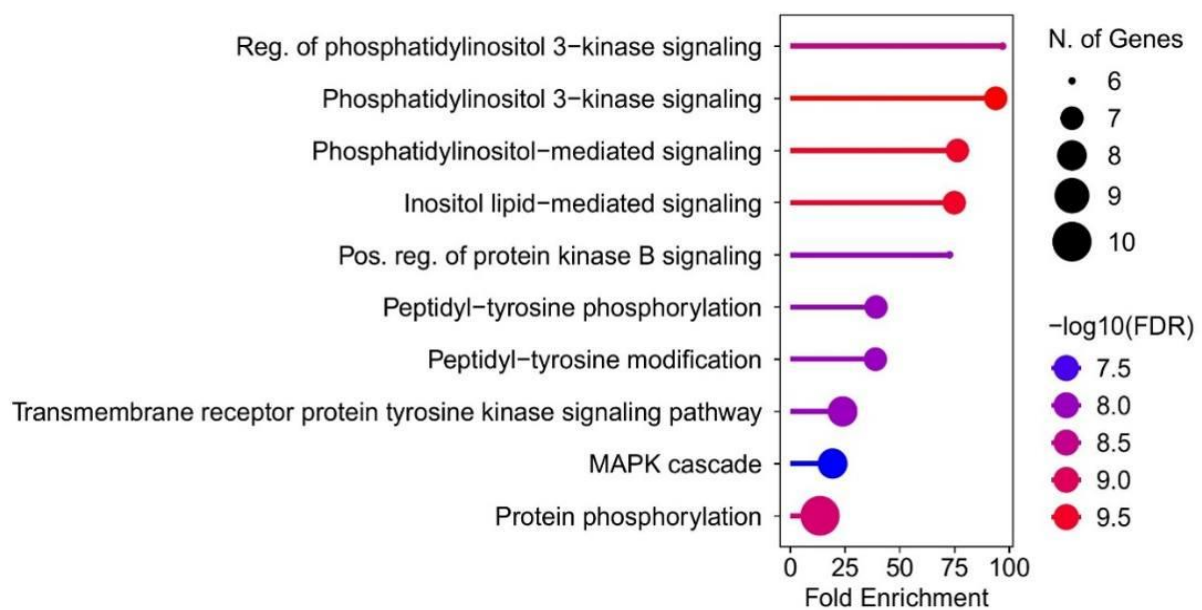


Figure 11: Biological Process (BP)

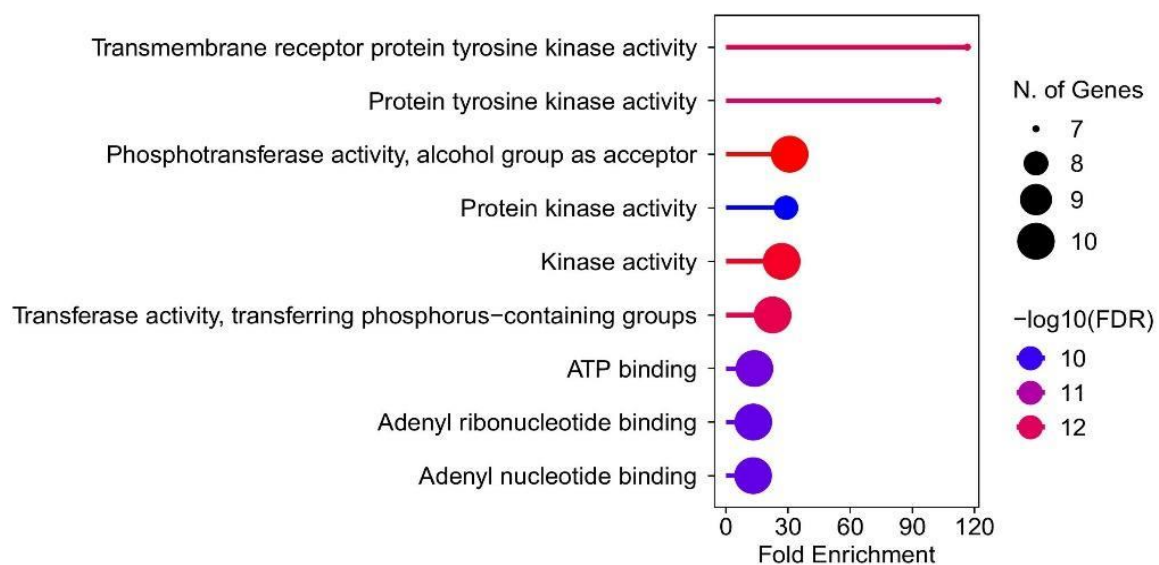


Figure 12: Molecular Function (MF)

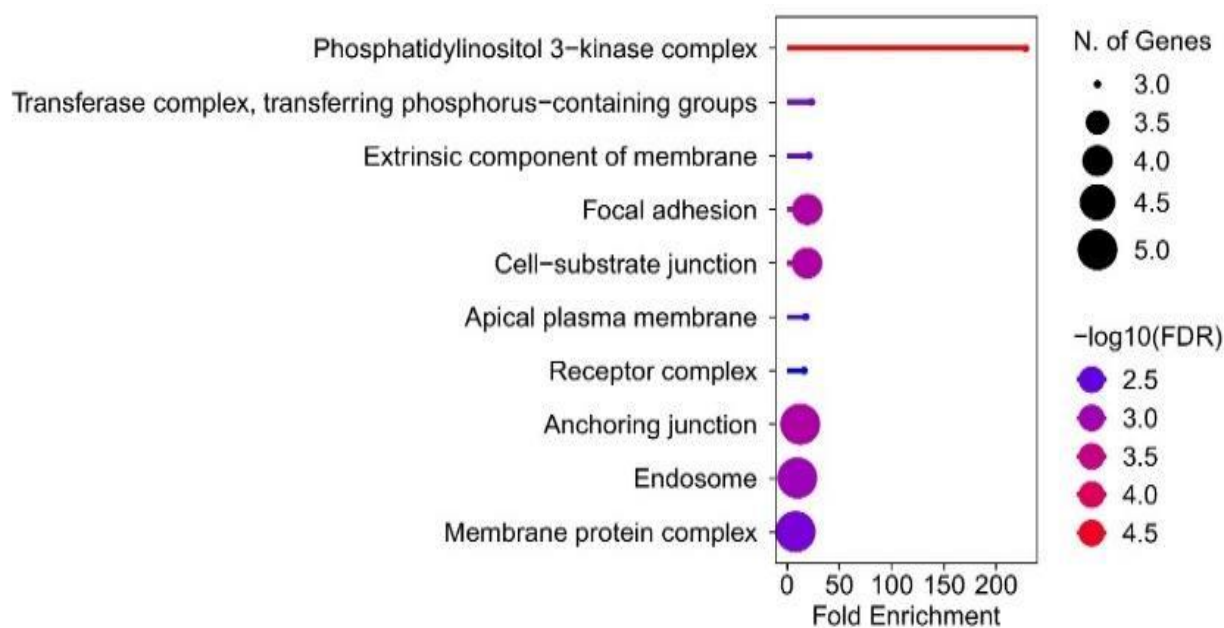


Figure 13: Cellular Component(CC)

MOLECULAR DOCKING

On the basis of the results obtained in the Swiss target prediction and network pharmacology, the therapeutic potential of the synthesized compounds against glioblastoma multiforme and Inflammation was investigated by using Molecular docking analysis.

According to the KEGG pathway enrichment analysis in Network pharmacology, the identified EGFR Tyrosine Kinase Inhibitor Resistance signaling pathway reveals,

- EGFR acts as the primary driver on the cell membrane, turning on downstream signals that force glioma cells to grow and multiply rapidly.
- To support this rapid growth, VEGFR triggers its own signaling pathway to promote angiogenesis, which sprouts new blood vessels to deliver food and oxygen to the glioblastoma mass.
- When inflammation occurs in the tumor microenvironment, it releases inflammatory signals like IL-6, which directly connect to and activate the Jak-STAT signaling pathway.
- JAK2 process these inflammatory and growth signals, causing the abnormal activation and pairing up of STAT3 proteins.
- This activated STAT3 moves straight into the cell's DNA, where it switches on genes that allow the cancer cells to migrate (motility) and protects them from dying (survival).
- Therefore, as demonstrated by in silico studies by blocking EGFR, VEGFR and JAK1 at the same time, the synthesized compounds shut down both the main growth engines and the inflammatory backup routes, trapping and destroying the glioblastoma cells.

MOLECULAR DOCKING PROFILING AGAINST TARGET RECEPTORS

Compound ID	RECEPTORS BINDING ENERGY (kcal/mol)		
	EGFR (kcal/mol)	VEGFR(kcal/mol)	JAK1 (kcal/mol)
3A	-7.6	-8.8	-8.0
3B	-7.3	-8.9	-8.3
3C	-7.3	-8.4	-7.7
3D	-7.7	-8.8	-8.2
3E	-8.3	-9.7	-9.0
3F	-7.6	-8.7	-8.3

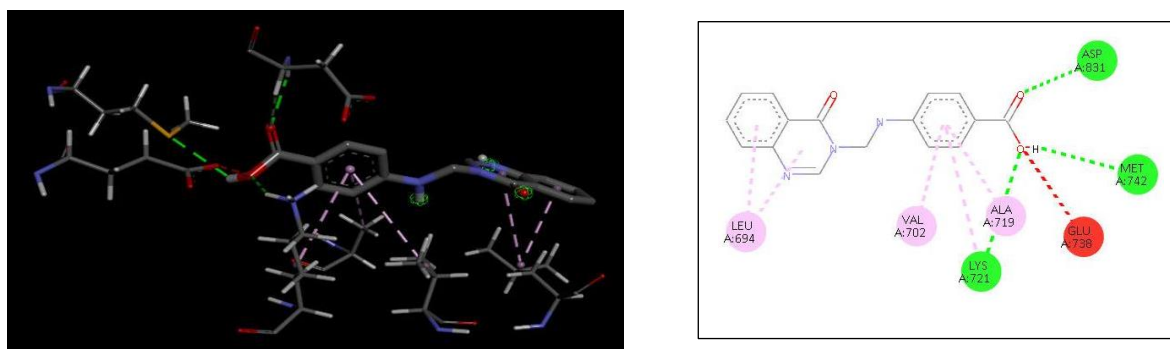
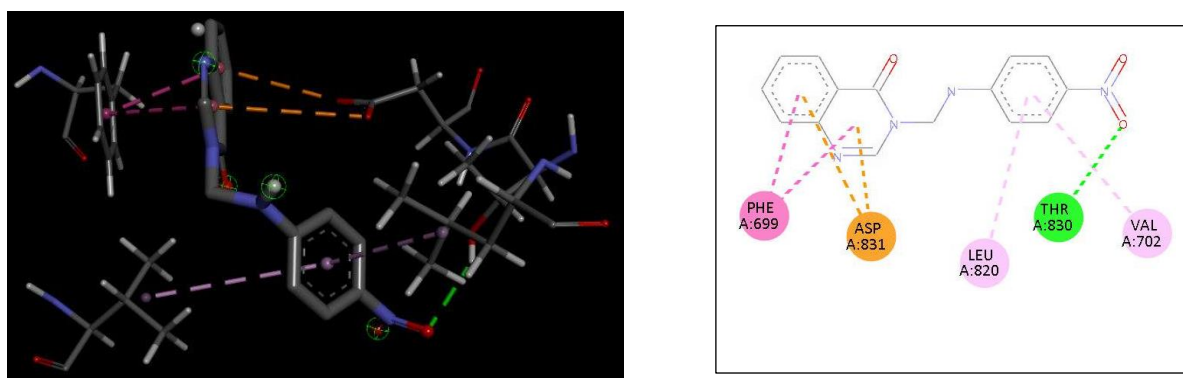
Compound ID	RECEPTORS BINDING ENERGY (kcal/mol)		
	EGFR (kcal/mol)	VEGFR(kcal/mol)	JAK1 (kcal/mol)
GEFITINIB	-7.2	-	-
SORAFENIB	-	-12.1	-
UPADACITINIB	-	-	-9.0

Table 5: Binding engeries of synthesized compounds of different receptor

The binding energies of the compounds and the reference drugs are summarized in Table 5. Crucially, the three reference standard drugs utilized in this comparative study represent distinct therapeutic classifications and pharmacodynamic categories. Gefitinib belongs to the class of classic EGFR tyrosine kinase inhibitors targeting primary oncogenic proliferation inputs, while Sorafenib acts as an anti-angiogenic multi-kinase inhibitor designed to starve the vascular support architecture of the tumor. Upadacitinib fall under the class of selective Janus Kinase inhibitors, primarily functioning as anti-inflammatory immunomodulators that shut down cytokine backup networks.

Epidermal Growth Factor Receptor (EGFR)

The comparative molecular docking study of the compounds 3A-3F to the EGFR was carried out in order to clarify the binding mode of the synthesized compounds. The 3D and 2D ligand–receptor interaction diagrams are shown in Figures 14–20.

**Figure 14: 3D and 2D ligand-receptor interactions of the compound 3A****Figure 15: 3D and 2D ligand-receptor interactions of the compound 3B**

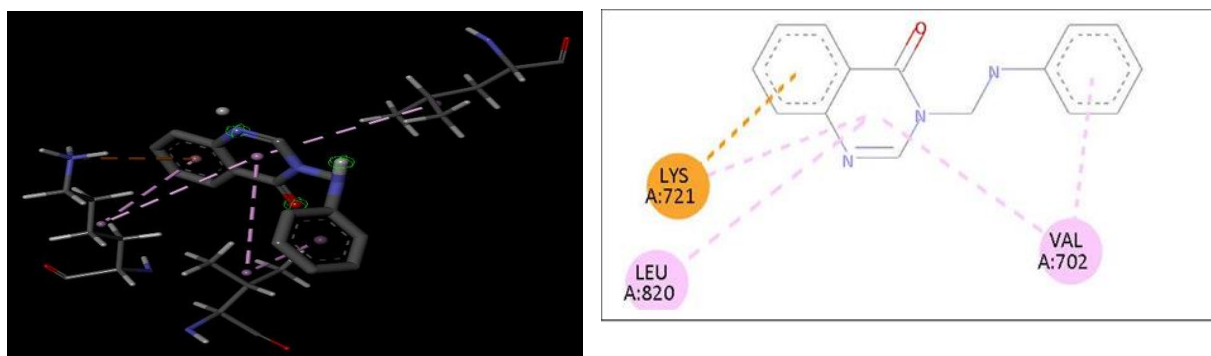


Figure 16: 3D and 2D ligand-receptor interactions of the compound 3C

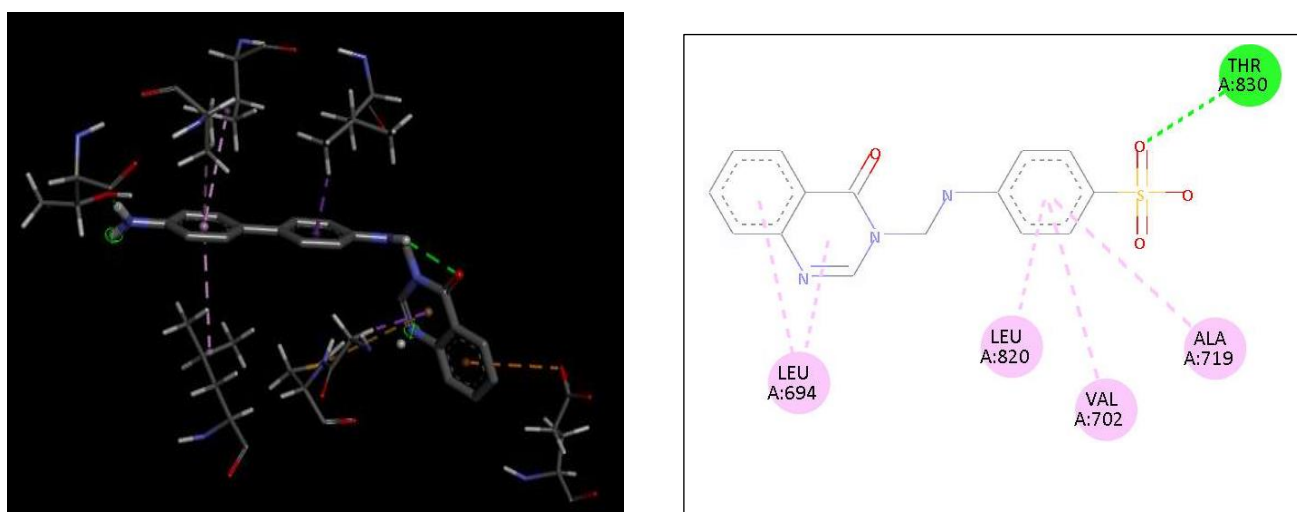


Figure 17: 3D and 2D ligand-receptor interactions of the compound 3D

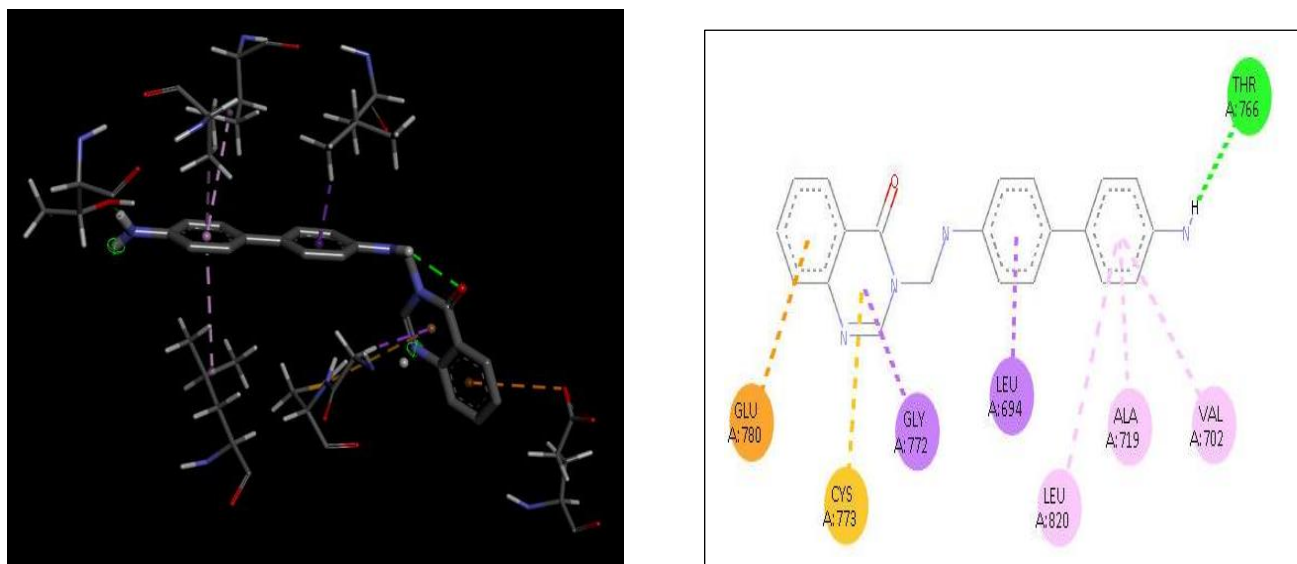


Figure 18: 3D and 2D ligand-receptor interactions of the compound 3E

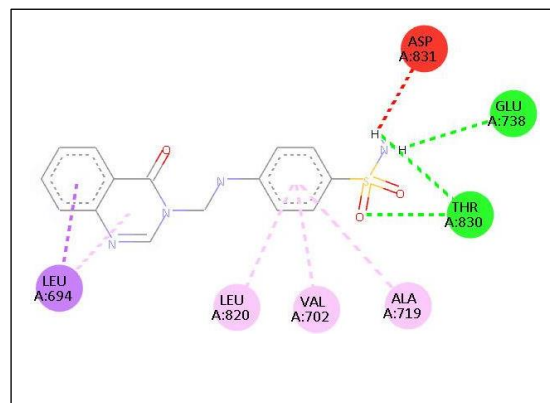
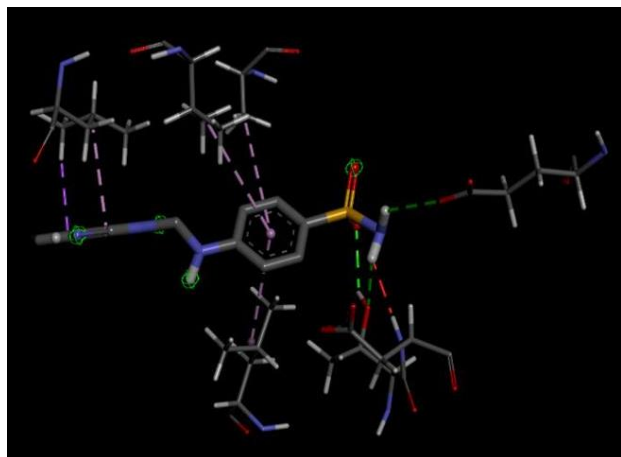


Figure 19: 3D and 2D ligand-receptor interactions of the compound 3F

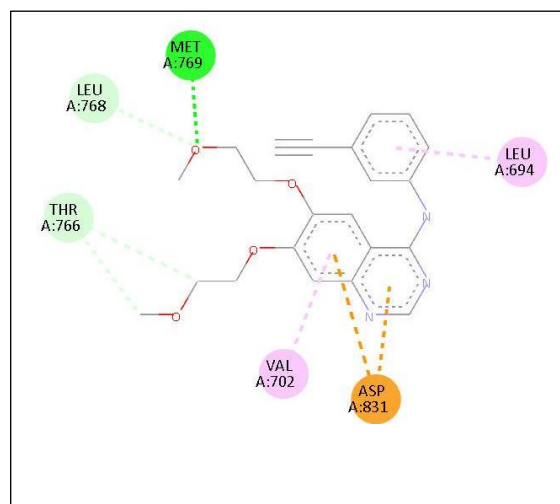
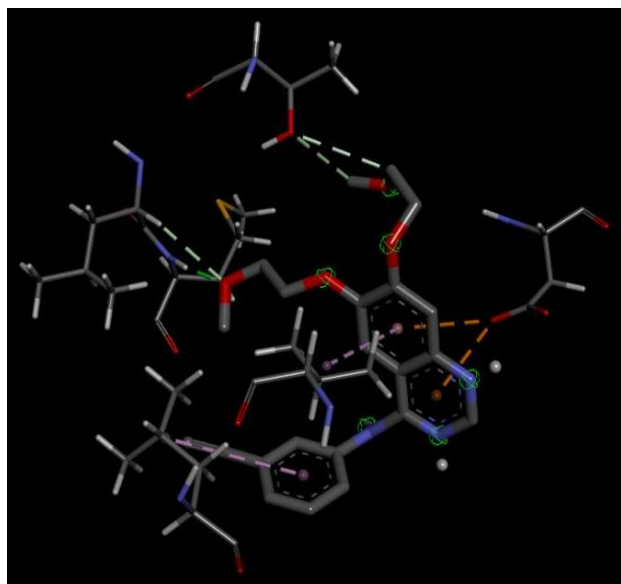


Figure 20: 3D and 2D ligand-receptor interactions of the compound GEFITINIB

The binding affinities of the synthesized ligands toward the selected Epidermal growth factor receptor structure (PDB ID: 1M17) were predicted via computational docking using the PyRx software.

Molecular docking studies of synthesized ligands with 1M17 PDB show that all the docked compounds bind with the receptor and exhibit free energy of binding value from -7.3 to -8.3 Kcal/mole (Table 5). All the synthesized compounds showed higher binding affinity than the standard drug gefitinib.

All the synthesized compounds bind in the active site and the simulated conformation exhibits various interactions with different amino acids by non-covalent interactions such as hydrophobic, hydrophilic, π - π interaction, and hydrogen bonding.

Among all the docked compounds, compound 3A shows a binding energy of about -7.6 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, specialized aromatic mapping, and a specific unfavorable contact across Chain A. Specifically, robust polar anchoring is achieved through conventional hydrogen bonds at the terminal carboxylic acid moiety involving Asp831 and Met742. This polar network is further supported by a localized π -donor hydrogen bond interaction between the central phenyl ring and Lys721, while a distinct unfavorable acceptor-acceptor contact is noted with Glu738 at the carboxylic acid group. Hydrophobic stabilization is mediated by coordinated multi-center aromatic mapping across the framework. The bicyclic core system forms localized π -interactions with Leu694, while the central phenyl ring is tightly secured within a hydrophobic pocket through coordinated π -contacts with Val702 and Ala719.

Compound 3B shows a binding energy of about -7.3 kcal/mol, and exhibits very high binding with 1M17 enzyme. . Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, distinct electrostatic anchoring, and localized hydrophobic mapping across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the terminal nitro group, which interacts

directly with Thr830. Electrostatic stabilization is significantly enhanced by a prominent π -anion interaction between the core bicyclic system and Asp831. Hydrophobic anchoring is further mediated by coordinated multi-center aromatic mapping across the framework, where the bicyclic core system forms localized π -interactions with Phe699, while the central phenyl ring and peripheral nitro group are secured within the pocket through coordinated π -alkyl/alkyl contacts with Leu820 and Val702.

Compound 3C shows a binding energy of about -7.3 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a localized binding network primarily driven by strong electrostatic anchoring and multi-center hydrophobic mapping across Chain A. Specifically, robust electrostatic stabilization is achieved through a prominent π -cation interaction involving Lys721 and the core aromatic ring framework. Hydrophobic anchoring is further mediated by coordinated π -network mapping across the molecular scaffold. The bicyclic core system forms localized π -interactions with Lys721 and Leu820, while the terminal phenyl ring and core segments are secured within the pocket through coordinated π -alkyl/alkyl interactions with Val702.

Compound 3D shows a binding energy of about -7.7 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding and a well-coordinated hydrophobic π -network across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the terminal sulfonic acid oxygen atom, which interacts directly with Thr830. Hydrophobic stabilization is heavily mediated by multi-center aromatic mapping across the molecular framework. The bicyclic core system forms localized π -interactions with Leu694, while the central phenyl ring is tightly secured within a hydrophobic pocket through coordinated π -contacts with Val702, Ala719, and Leu820.

Compound 3E shows a binding energy of about -8.3 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, distinct electrostatic anchoring, and an extensive hydrophobic π -network across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the terminal amine group, which interacts directly with Thr766. Electrostatic and specialized stabilization is heavily augmented near the bicyclic core framework by a π -anion interaction with Glu780, a π -sulfur interaction with Cys773, and a localized aromatic interaction with Gly772. Hydrophobic anchoring is further mediated by multi-center aromatic mapping across the framework, where the central phenyl ring forms a localized π -interaction with Leu694, while the terminal phenyl ring is tightly secured within the pocket through coordinated π -alkyl/alkyl contacts with Val702, Ala719, and Leu820.

Compound 3F shows a binding energy of about -7.6 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, an extensive hydrophobic π -network across Chain A, and a specific unfavorable contact. Specifically, robust polar anchoring is achieved through conventional hydrogen bonds formed by the terminal sulfonamide group, which interacts directly with GluA:738 and ThrA:830. These interactions are supported by a localized polar contact between the sulfone oxygen and ThrA:830, while a distinct unfavorable contact is noted between the sulfonamide moiety and AspA:831. Hydrophobic stabilization is heavily mediated by multi-center aromatic mapping. The bicyclic core system forms localized π -interactions with LeuA:694, while the central phenyl ring is tightly secured within a hydrophobic pocket through coordinated π -contacts with ValA:702, AlaA:719, and LeuA:820.

The standard ligand shows a binding energy of about -7.2 kcal/mol, and exhibits very high binding with 1M17 enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by directional hydrogen bonding, distinct electrostatic anchoring, and localized hydrophobic mapping across Chain A. Specifically, polar anchoring is achieved through a hydrogen bond interaction between Met769 and the methoxy oxygen atom, which is further supported by weak polar/carbon-hydrogen bond contacts involving Leu768 and Thr766 along the flexible ether side chains. Electrostatic stabilization is significantly enhanced by dual π -anion interactions between the core bicyclic ring system and Asp831. Hydrophobic anchoring is mediated by coordinated multi-center aromatic mapping across the framework, where the central bicyclic core forms a localized π -interaction with Val702, while the terminal substituted phenyl ring is secured within the pocket through a π -interaction with Leu694.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR)

The comparative molecular docking study of the compounds 3A-3F to the VEGFR was carried out in order to clarify the binding mode of the synthesized compounds. The 3D and 2D ligand-receptor interaction diagrams are shown in Figures 21–27.

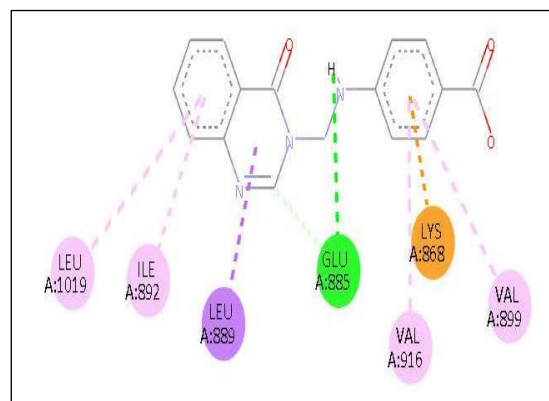
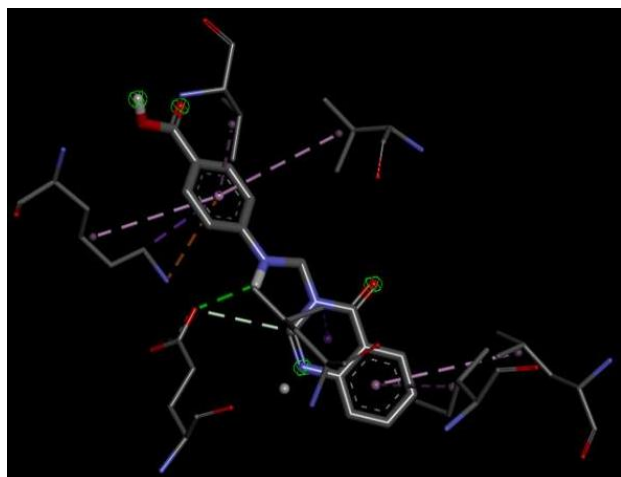


Figure 21: 3D and 2D ligand-receptor interactions of the compound 3A

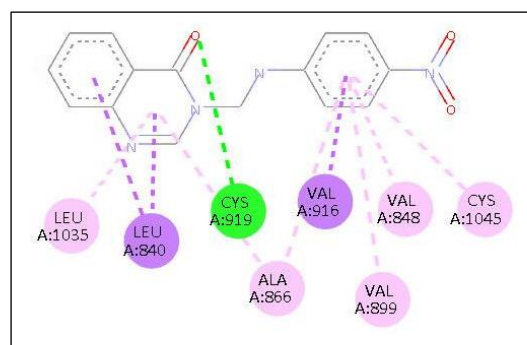
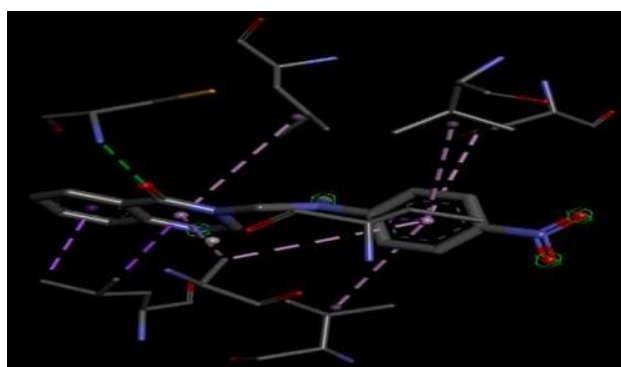


Figure 22: 3D and 2D ligand-receptor interactions of the compound 3B

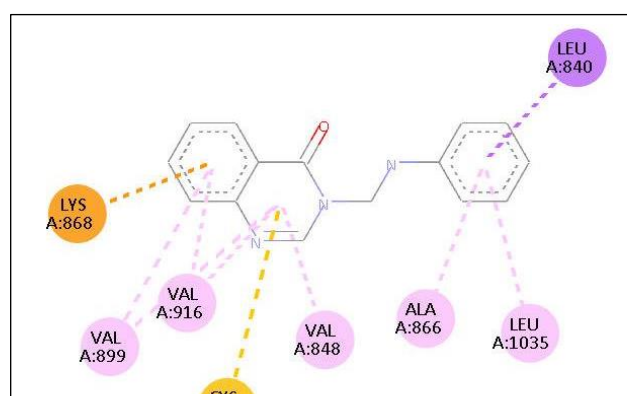
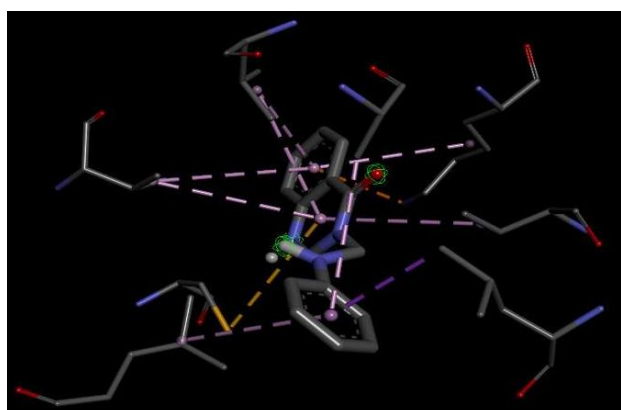


Figure 23: 3D and 2D ligand-receptor interactions of the compound 3C

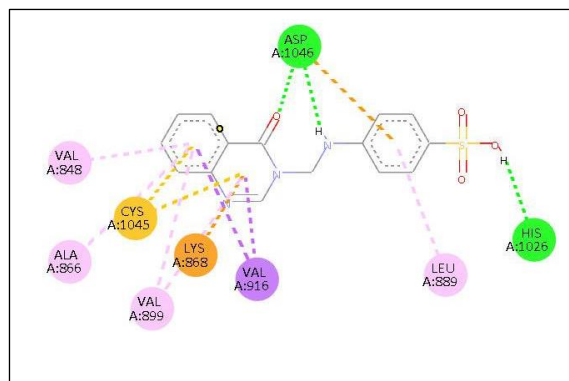
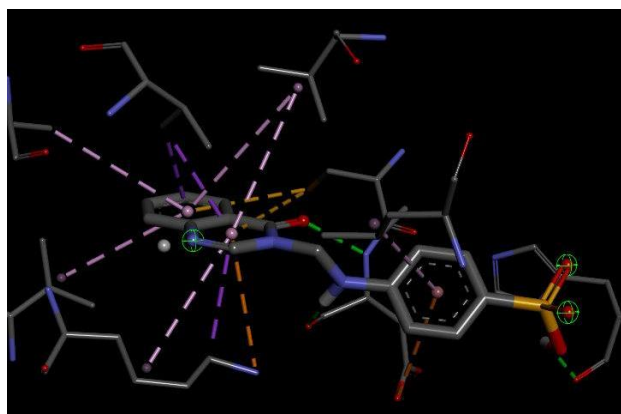


Figure 24: 3D and 2D ligand-receptor interactions of the compound 3D

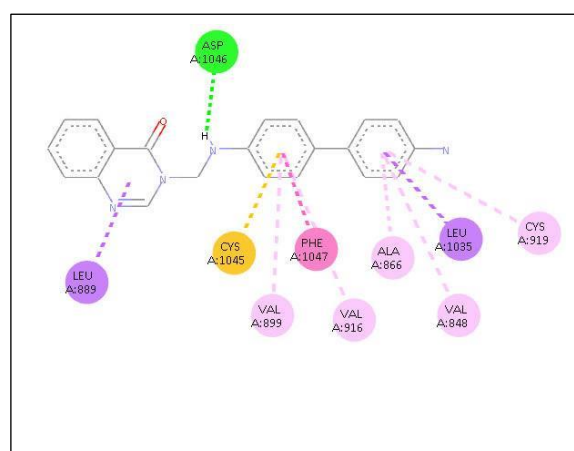
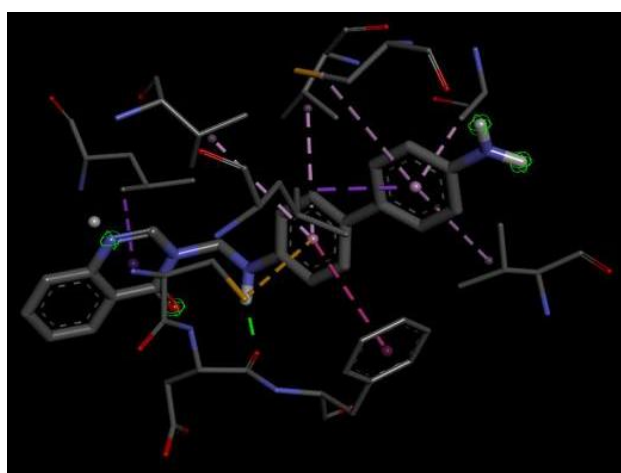


Figure 25: 3D and 2D ligand-receptor interactions of the compound 3E

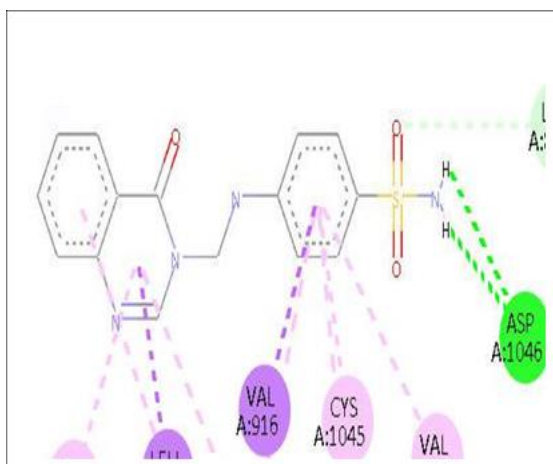
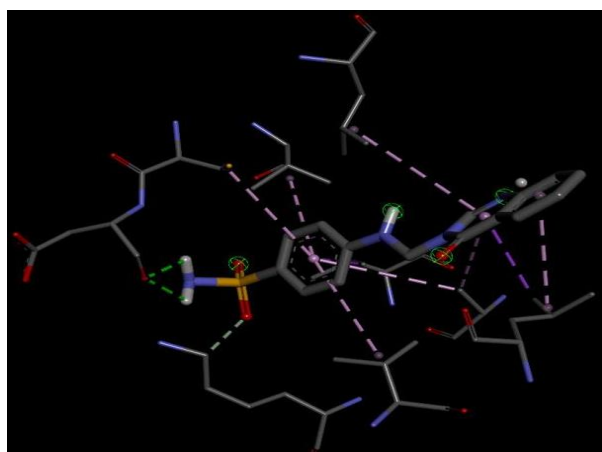


Figure 26: 3D and 2D ligand-receptor interactions of the compound 3F

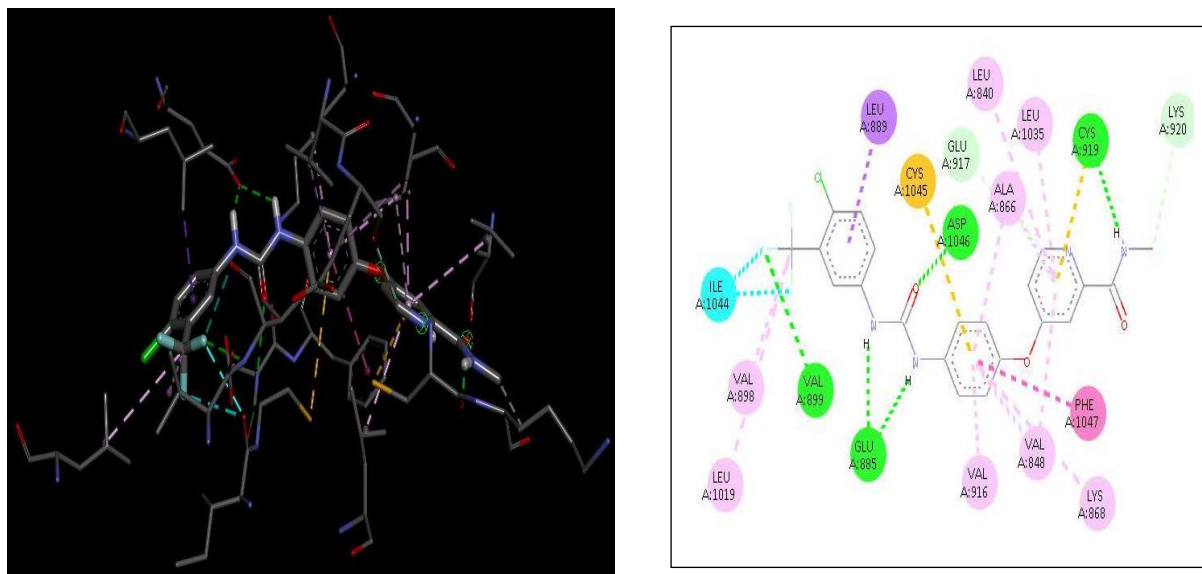


Figure 27: 3D and 2D ligand-receptor interactions of the compound SORAFENIB

The binding affinities of the synthesized ligands toward the selected Vascular endothelial growth factor receptor structure (PDB ID: 4ASD) were predicted via computational docking using the PyRx software.

Molecular docking studies of synthesized ligands with 4ASD PDB show that all the docked compounds bind with the receptor and exhibit free energy of binding value from -8.4 to -9.7 Kcal/mole (Table 5). All the synthesized compounds showed lower binding affinity than the standard drug sorafenib (-12.1 kcal/mol).

All the synthesized compounds bind in the active site and the simulated conformation exhibits various interactions with different amino acids by non-covalent interactions such as hydrophobic, hydrophilic, π - π interaction, and hydrogen bonding.

Among all the docked compounds, Compound 3A shows a binding energy of about -8.8 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, distinct electrostatic anchoring, and an extensive hydrophobic pocket mapping across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the amine linker group, which interacts directly with Glu885. Electrostatic stabilization is significantly enhanced by a prominent π -cation interaction between the central phenyl ring and Lys868. Hydrophobic anchoring is further mediated by coordinated multi-center aromatic mapping across both ring systems, where the bicyclic core forms localized π -interactions with Leu889, Ile892, and Leu1019, while the central phenyl ring is secured through coordinated π -contacts with Val899 and Val916.

Compound 3B shows a binding energy of about -8.9 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by directional hydrogen bonding and an extensive hydrophobic interaction network across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the core carbonyl oxygen atom, which interacts directly with Cys919. Hydrophobic stabilization is heavily mediated by multi-center aromatic mapping across the framework. The bicyclic core system forms localized π -interactions with Leu840 and Leu1035, while the central phenyl ring is tightly secured within a dense hydrophobic pocket through coordinated π -contacts with Val848, Ala866, Val899, Val916, and Cys1045.

Compound 3C shows a binding energy of about -8.4 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong electrostatic anchoring and an extensive hydrophobic interaction network across Chain A. Specifically, robust electrostatic stabilization is achieved through a prominent π -cation interaction between the bicyclic core system and Lys868, which is further supported by a localized π -sulfur interaction with Cys1045. Hydrophobic stabilization is heavily mediated by multi-center aromatic mapping across both ring frameworks. The bicyclic core forms localized π -interactions with Val848, Val899, and Val916, while the terminal phenyl ring is tightly secured within a hydrophobic pocket through coordinated π -contacts with Leu840, Ala866, and Leu1035.

Compound 3D shows a binding energy of about -8.8 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by multiple conventional hydrogen bonds, distinct electrostatic anchoring, and multi-center hydrophobic mapping across Chain A. Specifically, robust polar anchoring is achieved through direct hydrogen bonds involving the terminal sulfonic acid group with His1026, the core carbonyl oxygen atom with Asp1046, and the secondary amine linker with Asp1046. Electrostatic stabilization is significantly enhanced by a prominent π -cation interaction with Lys868, a π -anion interaction with Asp1046, and localized π -sulfur anchoring with Cys1045 near the bicyclic core. Hydrophobic stabilization is further mediated by coordinated multi-center mapping across the molecular scaffold, where the bicyclic core system forms localized π -interactions with Val848, Ala866, Val899, and Val916, while the central phenyl ring is secured through a π -interaction with Leu889.

Compound 3E shows a binding energy of about -9.7 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding, distinct specialized anchoring, and an extensive hydrophobic network across Chain A. Specifically, robust polar anchoring is achieved through a conventional hydrogen bond formed by the secondary amine linker group, which interacts directly with Asp1046. Near the central phenyl ring, specialized stabilization is mediated by a π -sulfur interaction with Cys1045 and a localized π - π stacked interaction with Phe1047. Hydrophobic anchoring is further extensively driven by multi-center mapping across the framework; the core bicyclic system forms a localized π -interaction with Leu889, the central phenyl ring contacts Val899 and Val916, and the terminal phenyl ring is tightly secured within the pocket through coordinated π -alkyl/alkyl interactions with Val848, Ala866, Cys919, and Leu1035.

Compound 3F shows a binding energy of about -8.7 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by strong directional hydrogen bonding and an extensive hydrophobic network across Chain A. Specifically, robust polar anchoring is achieved through a dual conventional hydrogen bond formed by the terminal sulfonamide group, which interacts directly with Asp1046. This polar network is further supported by a weak carbon-hydrogen bond/polar contact between the sulfone oxygen atom and Lys868. Hydrophobic stabilization is heavily mediated by multi-center aromatic mapping across the framework. The bicyclic core system forms localized π -interactions with Leu840 and Leu1035, while the central phenyl ring is tightly secured within a dense hydrophobic pocket through coordinated π -contacts with Val848, Ala866, Val899, Val916, and Cys1045.

The standard ligand shows a binding energy of about -12.1 kcal/mol, and exhibits very high binding with 4ASD enzyme. Molecular docking analysis revealed an extensive and highly stabilized binding network driven by multiple conventional hydrogen bonds, specialized halogen anchors, and multi-center hydrophobic mapping across Chain A. Specifically, robust polar anchoring is achieved through conventional hydrogen bonds, where the central amide linkers interact with Glu885 and Asp1046, and the terminal amide forms a direct contact with Cys919. These are supported by weak polar/carbon-hydrogen bond contacts involving Lys920, Glu917, and Val899. Halogen-mediated stabilization is prominent at the peripheral terminal, where the trifluoromethyl group forms halogen bonds with Val899 and Ile1044. Hydrophobic and specialized electrostatic forces heavily drive structural fit; the terminal chlorophenyl segment interacts with Leu889, while the central phenyl ring forms a π -sulfur interaction with Cys1045 alongside hydrophobic contacts with Val916 and Lys868. Furthermore, the heterocyclic core system completes the tight pocket fit via coordinated π -mediated interactions with Leu840, Ala866, Val848, Phe1047, Leu1035, and a π -sulfur contact with Cys919.

JANUS KINASE 1 (JAK1)

The comparative molecular docking study of the compounds 3A-3F to the JAK1 was carried out in order to clarify the binding mode of the synthesized compounds. The 3D and 2D ligand-receptor interaction diagrams are shown in Figures 28–34.

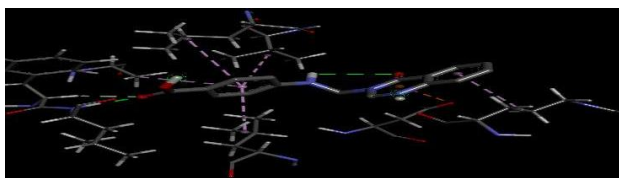


Figure 28: 3D and 2D ligand-receptor interactions of the compound 3A

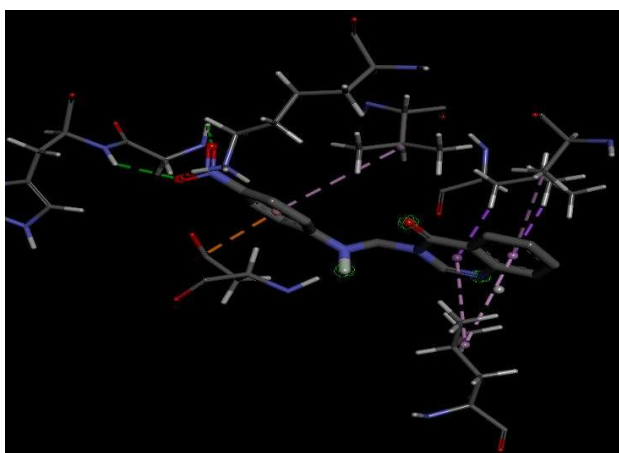
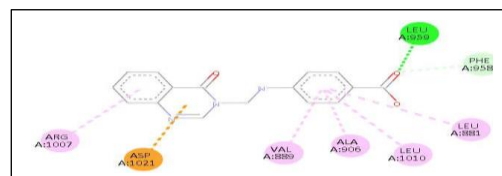
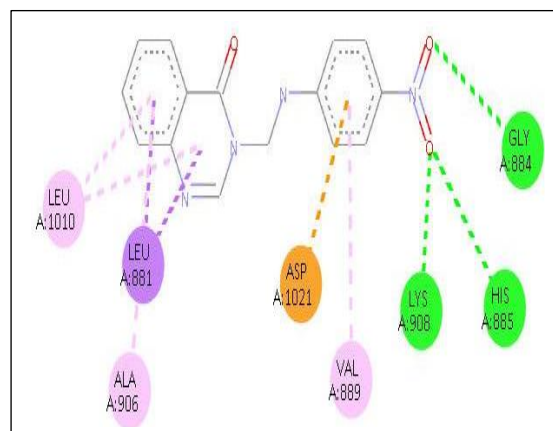


Figure 29: 3D and 2D ligand-receptor interactions of the compound 3B



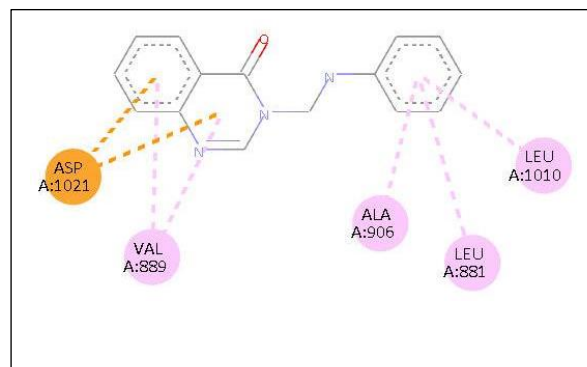
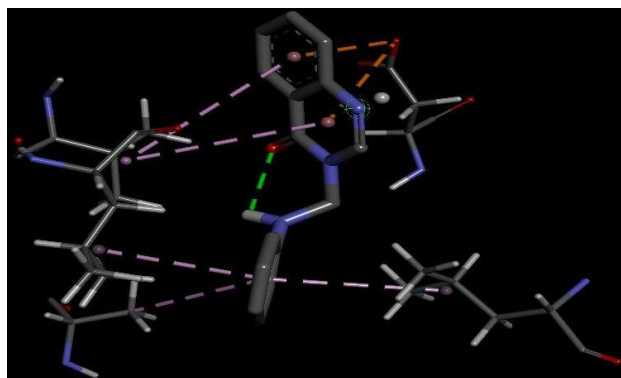


Figure 30: 3D and 2D ligand-receptor interactions of the compound 3C

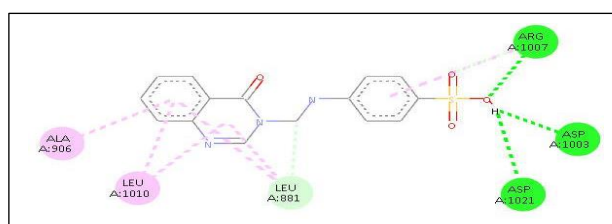
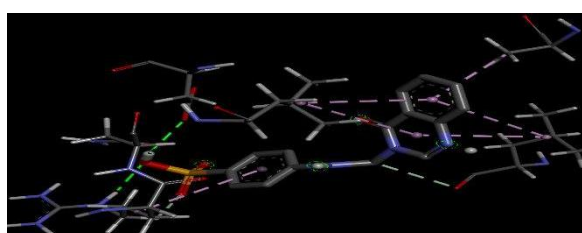


Figure 31: 3D and 2D ligand-receptor interactions of the compound 3D

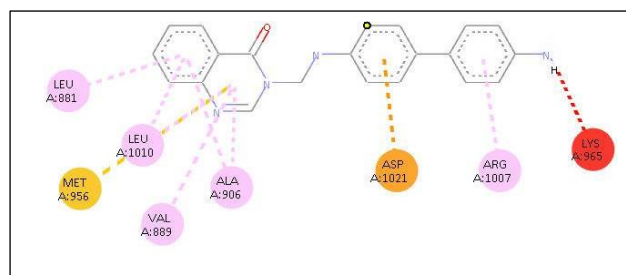
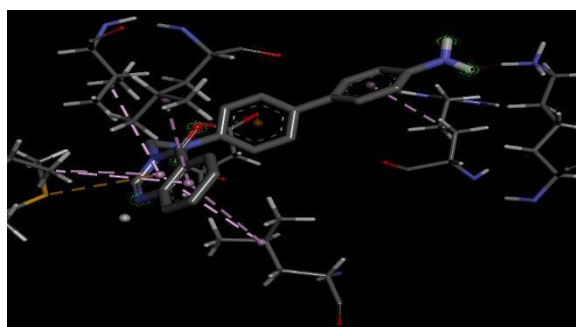


Figure 32: 3D and 2D ligand-receptor interactions of the compound 3E

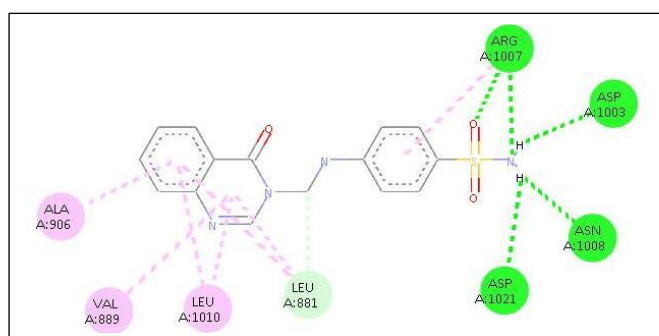
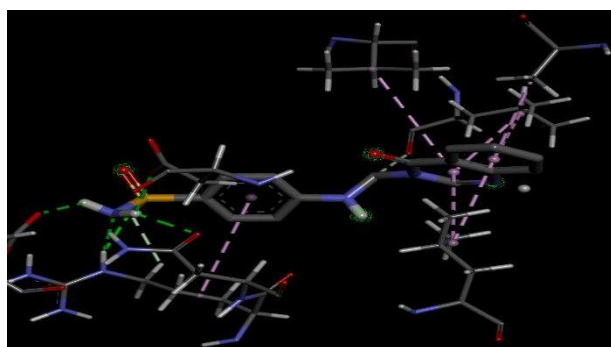


Figure 33: 3D and 2D ligand-receptor interactions of the compound 3F

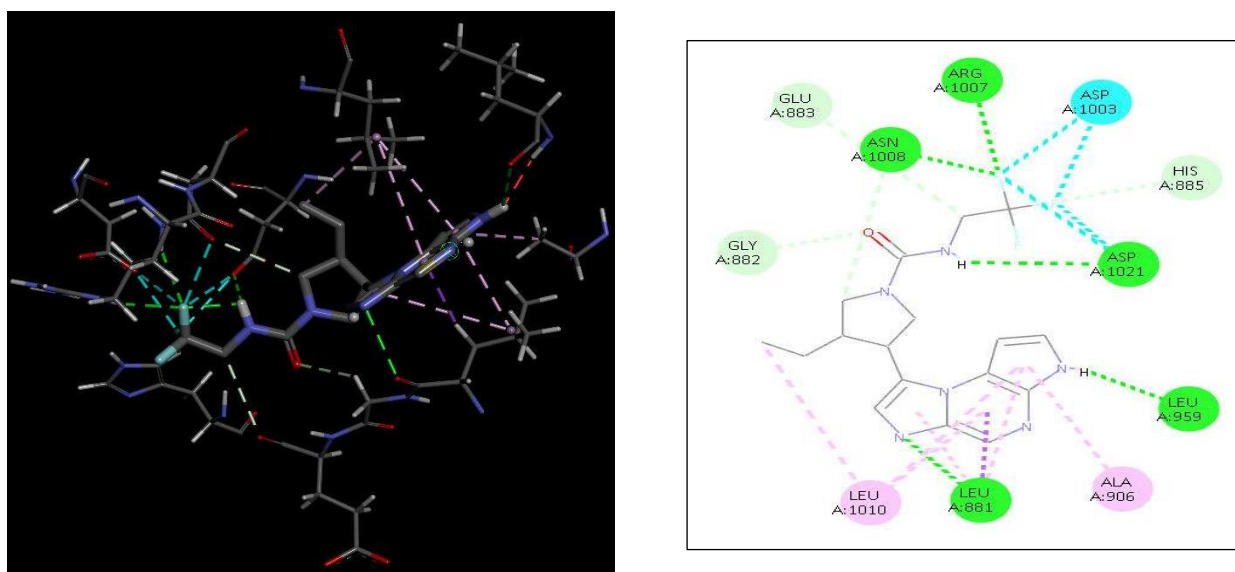


Figure 34: 3D and 2D ligand-receptor interactions of the compound UPADACITINIB

The binding affinities of the synthesized ligands toward the selected Janus kinase 1 enzyme crystal structure (PDB ID: 5E1E) were predicted via computational docking using the PyRx software.

Molecular docking studies of synthesized ligands with 5E1E PDB show that all the docked compounds bind with the receptor and exhibit free energy of binding value from -7.7 to -9.0 Kcal/mole (Table 5). Compound 3E showed binding affinity equal to that of the standard drug upadacitinib (-9.0 kcal/mol), whereas the other compounds showed lower affinity.

All the synthesized compounds bind in the active site and the simulated conformation exhibits various interactions with different amino acids by non-covalent interactions such as hydrophobic, hydrophilic, π - π interaction, and hydrogen bonding.

Among all the docked compounds, Compound 3A shows a binding energy of about -8.0 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), and robust polar anchoring is achieved through conventional hydrogen bonds formed by the terminal carboxylate group and a carbonyl oxygen atom, which interact directly with LeuA:959 and PheA:958. This is heavily supported by a coordinated π -anion contact involving AspA:1021 at the bicyclic core system, while the heterocyclic framework experiences stabilizing electrostatic mapping. Hydrophobic stabilization is mediated by targeted pocket mapping, where the central phenyl ring forms localized π -interactions with ValA:889, AlaA:906, LeuA:1010, and LeuA:881, while the terminal aromatic section is tightly secured through coordinated interactions with ArgA:1007.

Compound 3B shows a binding energy of about -8.3 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), and robust polar anchoring is achieved through conventional hydrogen bonds formed by the terminal nitro group and its oxygen atoms, which interact directly with GlyA:884, HisA:885, and LysA:908. This is heavily supported by a coordinated π -anion contact involving AspA:1021 at the central phenyl ring, while the heterocyclic framework experiences stabilizing pocket mapping. Hydrophobic stabilization is mediated by targeted pocket mapping, where the central phenyl ring forms a localized interaction with ValA:889, while the bicyclic core section is tightly secured through coordinated π -alkyl interactions with LeuA:1010 and LeuA:881.

Compound 3C shows a binding energy of about -7.7 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), where anchoring is uniquely driven by electrostatic and hydrophobic networks rather than conventional hydrogen bonding. This is heavily supported by multiple coordinated π -anion contacts involving AspA:1021 across the bicyclic core system, while the heterocyclic framework experiences stabilizing pocket mapping. Hydrophobic stabilization is mediated by targeted pocket mapping, where the terminal phenyl ring forms localized π -interactions with AlaA:906, LeuA:881, and LeuA:1010, while the bicyclic core section is tightly secured through a coordinated interaction with ValA:889.

Compound 3D shows a binding energy of about -8.2 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), and robust polar anchoring is achieved through conventional hydrogen bonds formed by the terminal sulfonic acid group, which interact directly with AsnA:1008, AspA:1021, and ArgA:1007. This is heavily supported by a carbon-hydrogen bond contact involving LeuA:881 near the central linker framework, while the heterocyclic core experiences stabilizing pocket mapping. Hydrophobic stabilization is mediated by targeted pocket mapping, where the bicyclic core system is tightly secured through coordinated π -alkyl interactions with AlaA:906 and LeuA:1010.

Compound 3E shows a binding energy of about -9.0 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), where anchoring at the distal end involves a localized contact between the terminal amino group and LysA:965. This is heavily supported by a coordinated π -anion contact involving AspA:1021 at the central phenyl ring, while the bicyclic core system experiences a specialized π -sulfur interaction with MetA:956. Hydrophobic stabilization is mediated by targeted pocket mapping, where the terminal aromatic section forms a localized interaction with

ArgA:1007, while the bicyclic framework is tightly secured through coordinated π -alkyl interactions with LeuA:881, LeuA:1010, ValA:889, and AlaA:906.

Compound 3F shows a binding energy of about -8.3 kcal/mol, and exhibits very high binding with the Chain A sub-unit of the JAK1 enzyme (5E1E), and robust polar anchoring is achieved through conventional hydrogen bonds formed by the terminal sulfonamide group and a sulfone oxygen atom, which interact directly with AspA:1003, AsnA:1008, AspA:1021, and ArgA:1007. This is heavily supported by a carbon-hydrogen bond contact involving LeuA:881 near the central linker framework, while the heterocyclic framework experiences stabilizing pocket mapping. Hydrophobic stabilization is mediated by targeted pocket mapping, where the bicyclic core system is tightly secured through coordinated π -alkyl interactions with AlaA:906, ValA:889, and LeuA:1010, while the central aromatic section forms localized interactions with ArgA:1007.

The standard ligand shows a binding energy of about -9.0 kcal/mol, and exhibits very high binding with 5E1E enzyme. Molecular docking analysis revealed a highly stabilized binding network driven by directional hydrogen bonding, distinct halogen anchoring, and localized hydrophobic mapping across Chain A. Specifically, polar anchoring is achieved through a hydrogen bond interaction between LeuA:959 and the tricyclic core amine group, alongside a stable contact between AspA:1021 and the amide linker, which is further supported by weak polar/carbon-hydrogen bond contacts involving GlyA:882, GluA:883, and AsnA:1008 along the framework. Halogen-mediated stabilization is significantly enhanced by multi-center network interactions between the terminal fluorinated group and AspA:1003, AsnA:1008, ArgA:1007, and HisA:885. Hydrophobic anchoring is mediated by coordinated multi-center mapping across the framework, where the tricyclic heterocyclic core forms localized π -interactions with LeuA:881 and AlaA:906, while the terminal aliphatic side chain is secured within the pocket through an alkyl interaction with LeuA:1010.

Discussion

The six N-Mannich base derivatives satisfied all of Lipinski's criteria, with molecular weights below 350 g/mol and Log P values of 2.24–3.37, which supports their potential as orally active, drug-like molecules. The pkCSM predictions showed good intestinal absorption for most compounds (HIA 91–98% for 3A, 3B, 3C and 3E), with the lowest value for 3D (57.8%). The predicted logBB values (–0.20 to –0.82) are above the –1.0 cut-off for poor brain distribution but below the 0.3 threshold for ready penetration, so brain exposure is expected to be moderate, with 3E having the most favorable value (–0.20). Several compounds were predicted to inhibit CYP3A4 and CYP1A2, which should be checked experimentally because of the risk of drug–drug interactions. ProTox-II predicted no hepatotoxicity, carcinogenicity, mutagenicity or cytotoxicity, and the predicted LD₅₀ was lowest for 3C (255 mg/kg), which was also the only compound flagged for immunogenicity.

The network pharmacology analysis supports a multi-target mechanism. The 126 shared targets formed a densely connected network (PPI enrichment $P < 1.0 \times 10^{-16}$), and EGFR, JAK2, ERBB2, the PIK3C isoforms and JAK1 were among the top hub genes. KEGG enrichment placed these targets in the EGFR tyrosine kinase inhibitor resistance, JAK-STAT and PI3K-Akt pathways, which link growth-factor signaling to inflammatory signaling in glioblastoma. This overlap is the reason EGFR, VEGFR and JAK1 were chosen for docking, because blocking one pathway alone may leave bypass routes open.

In the docking study, all six compounds bound the three targets with favorable energies, and 3E gave the lowest energy for each (EGFR –8.3, VEGFR –9.7 and JAK1 –9.0 kcal/mol). For EGFR, every compound scored better than gefitinib (–7.2 kcal/mol), and for JAK1, 3E matched upadacitinib (–9.0 kcal/mol). For VEGFR, all compounds scored well below sorafenib (–12.1 kcal/mol), so their anti-angiogenic potential is likely weaker than that of the reference drug. The binding poses were stabilized by hydrogen bonds, electrostatic contacts and hydrophobic interactions with residues in the active sites. Docking scores are only estimates of binding and do not prove inhibition, and ADME and toxicity values are predictions. The activity of 3E therefore needs to be confirmed by enzyme and cell-based assays and, ideally, by molecular dynamics simulations.

CONCLUSION

This study used network pharmacology and molecular docking to evaluate six N-Mannich base quinazolin-4(3H)-one derivatives as multi-target agents against glioblastoma and inflammation. The compounds met Lipinski's criteria and showed acceptable predicted ADME and toxicity profiles. The network analysis identified EGFR, JAK1 and related PI3K-Akt and JAK-STAT signaling as key shared targets. Compound 3E gave the best docking energies against EGFR, VEGFR and JAK1 and is the most promising lead. These are in silico findings, and further in vitro and in vivo studies are needed to confirm the activity and safety of this compound.

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