RESEARCH ARTICLE

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Quinoxaline as Dual Modulators of Apoptotic Regulators Bcl-2 and Bax: A Combined *In Vitro* and *In Silico* Anticancer Approach

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Abstract

Objective: This study aimed to evaluate the antioxidant potential of quinoxaline and investigate its molecular interactions with cancer-related proteins through computational docking. **Methods:** Antioxidant activity of quinoxaline was assessed using DPPH, FRAP, ABTS, hydrogen peroxide, superoxide, and reducing power assays at varying concentrations, and IC_{50} values were calculated. Molecular docking studies were performed to examine the interactions of quinoxaline with cancer-associated proteins, including epidermal growth factor receptor (EGFR), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), and β-actin. **Results:** Antioxidant assays showed a concentration-dependent increase in inhibitory activity, with IC_{50} values of 130.446 µM (DPPH), 151.343 µM (FRAP), 171.551 µM (ABTS), 108.194 µM (H_2O_2), 104.592 µM (superoxide), and 95.893 µM (reducing power assay). Molecular docking analysis revealed that quinoxaline exhibited strong binding affinity with the anti-apoptotic Bcl-2, suggesting potential inhibition of its function. Additionally, favorable interactions with the pro-apoptotic Bax were observed, indicating a possible dual mechanism of apoptosis induction. **Conclusion:** Quinoxaline demonstrated significant antioxidant activity and potential pro-apoptotic effects by targeting key apoptotic regulators. The docking results suggest that quinoxaline could inhibit anti-apoptotic Bcl-2 while promoting the activity of the pro-apoptotic Bax, thereby inducing apoptosis and highlighting its potential as a promising anticancer agent.

Keywords: Antioxidant- Cancer- Docking studies- EGFR- Quinoxaline

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Introduction

Cancer is defined as any unchecked cell proliferation that takes over and damages nearby cells and tissue. All live cells in the body, regardless of age and gender, are susceptible to cancer. There are several contributing factors for the cause of cancer and the disease process varies depending on the location and immune system of a particular body [1, 2]. The biggest solitary cause of cancer death is using tobacco products. Approximately half of the 4.9 million fatalities from all causes in 2000 were in industrialised nations, and the other half were in developing nations. 9 million people will die from tobacco-related causes in 2020, with 7 million of those deaths occurring in emerging nations, if the tobacco usage becomes more [3]. Women's fatality rose by twofold while men's fatality jumped by threefold during the course of 40 years. The primary cause of cancer-related sickness and death is altered exposure to risk factors. According to the World Health Organization, cancer kills more people globally than heart disease [4]. According to world health organisation studies, lifestyle factors like diet, smoking, and alcohol usage may be responsible for 35% of cancer-related deaths globally. Furthermore, the main risk factors in nations with low, medium, and high incomes include infections, exposure to UV light, environmental tobacco smoking, hormone replacement therapy, physical and chemical factors, and radioactive exposure [5, 6]. There are various kinds of cancer, but the two most common ones that cause more mortality and morbidity are liver and breast cancer. Liver cancer is a major worldwide public health issue. According to estimates from 2020, liver

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cancer is the third leading cause of cancer-related mortality and ranks sixth in incidence among all cancers [7]. It is estimated that liver cancer claimed the lives of 760,000 people worldwide in 2022 and caused 866,136 new cases of the disease. It is estimated that the number of new cases and deaths from liver cancer will climb by further 50% over the next 20 years [8]. Breast cancer remains one of the most frequently diagnosed malignancies in women across the globe, affecting individuals of various age groups. Its development is influenced not only by hormonal and reproductive elements but also by environmental exposures. The risk factors include an early age at the onset of menstruation, weight gain after menopause, and extended use of estrogen-based hormone therapy [9]. According to global cancer statistics, more than 2.3 million new cases of breast cancer were reported in 2020, resulting in nearly 685,000 deaths worldwide [10]. The standard and advised methods for addressing cancer are eliminating the tumors surgically, followed by chemotherapy and radiation therapy. Surgical intervention is considered the most effective treatment option when the disease is detected in its early stages. However, radiation therapy may pose a risk of damaging surrounding healthy tissues. In addition to this, chemotherapeutic drugs harm quickly proliferating and growing healthy cells, despite the fact that chemotherapy has decreased morbidity and mortality [11]. Therefore, the cancer prevention is still a significant challenge in terms of morbidity and mortality, despite numerous types of research and advancements in cancer therapy. The search for anti-cancer drugs has involved enormous scientific efforts. Because of this, natural products that have been investigated for a long time have been shown to have pharmacologic activity and to be safe when exposed for an extended period [12]. The main component in the battle against cancer is antioxidants found in plants. All the main components of cells, including DNA, proteins, and cell membranes, can be harmed by free radicals, which are extremely reactive molecules. In addition to causing abrupt alterations, DNA damage can also result in cancer development [13, 14]. In addition to this the limitations of the traditional chemotherapeutic technique include improper drug metabolism, challenges with selecting concentrations, lack of preciseness, and predominant side effects [15]. Pharmaceutical discovery related to drugs considered as a multi-step platform that allows for the selection of a particular chemical compound with the required biological action on the drug target, which later becomes a candidate drug for drug development. This platform evaluates biological targets and chemical substances from several perspectives utilising a variety of methodologies. In this docking studies or molecular docking studies are the most potential computational methods which are used to understand the interactions between target protein and ligand (Small molecule). Further, we can also estimate the drug potential or strength that can bind to specific targets or enzymes. Most importantly finding interactions and understanding the drug potential related binding specific targets plays a crucial role in the drug discovery. The developments of new drugs are highly expensive and majorly time taking process. Compound screening tests are one of the previously mentioned methods that can help with hit authentication, confirmation, development of leads, and customisation in addition to evaluating the compounds' effect on the therapeutic target. Techniques like virtual screening are being utilised more and more in drug discovery and design projects as technology develops and computational science is incorporated with biological and pharmaceutical research [16-19].

Targeting key molecular pathways involved in tumor growth and survival is essential for the development of effective anti-cancer therapies. Benzopyrazine, also referred to as quinoxaline, is an isostere of naphthalene in which nitrogen atoms have been substituted for carbon atoms 1 and 4. One significant class of heterocycles seen in natural product is quinoxaline [20, 21]. Quinoxaline ($C_8H_6N_2$), identified as a prominent compound in the mangrove which represents an important structural framework in drug discovery due to its wide biological potential [22]. Quinoxaline display diverse pharmacological activities such as antibacterial, antifungal, antiviral, anticancer and anti-metastatic [21, 22]. Moreover, quinoxaline analogues show therapeutic promise against cardiac disorders, tuberculosis and parasitic infections [21]. Many of these derivatives and their synthetic methods are patented globally, reflecting their importance in medicinal chemistry.

The epidermal growth factor receptor (EGFR) consists of three main parts: an extracellular domain that binds to ligands, a transmembrane segment, and an intracellular domain with tyrosine kinase activity. Tyrosine residues in the receptor's cytoplasmic domain get autophosphorylated, the cellular kinase domain is activated, and the receptor dimerises when a ligand binds to the extracellular phase of the EGFR [23]. Therefore, In the present study, we explored the antioxidant potential and molecular docking interactions of quinoxaline, a heterocyclic compound recognized for its broad pharmacological activities, against several cancer related proteins, including EGFR, B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax) and β-actin.

Materials and Methods

Chemicals

All the chemicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Ascorbic acid, Hydrogen peroxide (H_2O_2), Phosphate buffer (pH 7.4), TPTZ (2,4,6-tripyridyl-s-triazine), Ferric chloride, Acetate buffer (pH 3.6), ABTS, Potassium persulfate, NADH (Nicotinamide adenine dinucleotide, reduced form), NBT (Nitroblue tetrazolium), Trichloroacetic acid (TCA), Quinoxaline 98% purity & Ferric & Ferric chloride are procured from Sigma-Aldrich and all the chemicals are analytical grade which are ready to use.

Selection of Receptor

In this the protein and its receptor was selected depending on major role in binding and in pathogenesis of the target illness (Cancer). The Protein Data Bank (http://www.rcsb.org) was used and tried for the 3D structure of the receptor binding sites of EGFR Protein Data Bank

(PDB ID:4ZAU) (Figure 2a), Bcl2 (PDB ID:6QGH) (Figure 2b), Bax (PDB ID:5W62) (Figure 2c) and Beta actin (PDB ID:2BTF) (Figure 2d). This process and the structure was considered depending on the resolution, ligand availability and applicability to the biological mechanism being studied.

Selection of Ligand

In contemporary pharmaceutical research, protein–ligand docking is essential for drug development and discovery. Docking methods use sampling and ranking to estimate the ligand-receptor complex's structure. They start by taking a sample of the ligands' conformation in a receptor's active region. Additionally, they compute the binding energy and rank each of the resulting poses according to particular scoring formulas. As a result, docking algorithms can mimic the ideal ligand orientation when it binds to a protein receptor [24]. The 3D structures of bioactive compounds namely quinoxaline is shown in figure 3 were collected from Pubchem (http://pubchem.ncbi.nlm.nih.gov), a compound database. The collected Structure Data File (SDF) files of identified bioactive compound from the PubChem database were converted into Protein Data Bank (PDB) format using the EduPymol version 1.7.4.4.

Antioxidant Assays

The test sample's antioxidant (free radical scavenging activity) potential was examined using a variety of in vitro antioxidant assays, including the DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)radical cation decolourisation assay, hydrogen peroxide scavenging assay, superoxide anion radical scavenging assay, and reducing power assay for radicals. Analysis of various concentrations at 20, 40, 80, 160 and 320 µg/mL was done for each assay. The current antioxidant assays are conducted in triplicate with ascorbic acid as standard and the mean ± standard deviation (SD) was used to represent the results.

DPPH Assay

A 0.3 mM concentration of the DPPH standard (reagent stock) solution was made in 1000 millilitres of methanol for the DPPH assay. In order to create the sample stock solution, 0.01g was dissolved in one millilitre of the appropriate solvent (100 mg/ml). From there, several concentrations, including 20, 40, 80, 160, and 320 µg/ml, were created. Two millilitres of DPPH reagent were combined with one millilitre of each sample solution, which was then kept in a dark location and given 30 minutes to react at room temperature. DPPH is reduced and turns from deep violet to bright yellow when it combines with antioxidant chemicals found in plant extracts. After a 30-minute incubation period, the absorbance was recorded at 517 nmusing aUV-Visible spectrophotometer. The percentage of radical scavenging(antioxidant) activity was calculated using standard equations. For the control, one millilitres of solvent was mixed with two millilitres of the DPPH solution, as described by [25].

FRAP Assay

One millilitre of methanol (100 mg/ml) is combined with 0.01 gm of sample, and various concentrations, including 20, 40, 80, 160, and 320 µg/ml, are created from this mixture. Add 3.6 mL of FRAP solution to 0.4 mL of distilled water, and then incubate both solutions for 10 minutes at 37˚C. The reaction mixture's absorbance was measured at 593 nm. The absorbance values were determined as for sample solutions for five different concentrations of $FeSO_4.7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM) in order to generate the calibration curve [26].

ABTS Assay

7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) reacted to create the ABTS + cation radical, which was then left at room temperature for 12–16 hours in the dark before use. The absorbance of 0.700 at 734 nm was then achieved by diluting the ABTS·+ solution with methanol. Absorbance was determined 30 minutes after 5 µl (20, 40, 80, 160, and 320 µg/ml) of samples were added to 3.995 ml of diluted ABTS·+ solution. The percentage blockage of absorbance was measured at 734 nm [27].

H_2O_2 Assay

0.2 M sodium hydroxide and about 0.2 M potassium dihydrogen phosphate solutions were made. The volumetric flask (capacity: 200 ml)was filled with 50 ml of potassium dihydrogen phosphate solution, followed 39.1 ml of 0.2 M sodium hydroxide solution was added. The volume was then adjusted to 200 ml using distilled H_2O to create phosphate buffer at (pH-7.4). An equivalent volume of hydrogen peroxide was mixed with 50 millilitres of phosphate buffer solution to create free radicals. The reaction was then allowed to finish for five minutes at room temperature. The absorbance at 230 nm was assessed against a blank, and the percentage of scavenging was calculated when one millilitre of sample (20, 40, 80, 160 and 320 µg/ml) was added to 0.6 millilitres of hydrogen peroxide solution [28].

Superoxide free radical scavenging Assay

The reduction of nitro blue tetrazolium (NBT) to a blue-coloured formazan product serves as an indicator of superoxide anion generation, which occurs during the oxidation of NADH in the riboflavin–NADH system. In this assay, varying concentrations of the test sample (20, 40, 80, 160, and 320 µg/ml) were prepared by adding 0.02 ml of each concentration to test tubes containing 0.05 ml of riboflavin, 0.2 ml of EDTA solution, and 0.1 ml of NBT solution. The total volume of each reaction mixture was brought to 2.64 ml using phosphate buffer. Following preparation, the mixtures were exposed to fluorescent light for five minutes, after which their absorbance was recorded at 560 nm using DMSO as the blank. The change in optical density (OD) was measured again after a 30-minute incubation to assess the level of NBT reduction [29].

Reducing Power Assay

Various concentrations of the test sample (20, 40, 80, 160, and 320 µg/mL) were each mixed with 0.050 mL of 0.2 M phosphate buffer (pH 6.6) and 0.2 mL of a 1% potassium ferricyanide solution. The mixtures were incubated in a water bath set at 50°C for 20 minutes. After incubation, 0.25 mL of trichloroacetic acid was added to each tube, and the solutions were then centrifuged at 1000 rpm for 10 minutes at room temperature. From the resulting supernatant, 0.5 mL was collected and combined with an equal volume of deionised water and 0.1 mL of 0.1% ferric chloride ($FeCl_3$) solution. The absorbance of the final reaction mixture was recorded at 700 nm to evaluate the reducing power of the samples [30].

Protein-Ligand Docking

The protein-ligand complex's preferred shape was assessed using molecular docking analysis. Without its two essential elements the target protein and the ligand the docking phase is pointless. Target proteins for the docking investigation were EGFR (PDB ID:4ZAU) (Figure 2a), Bcl2 (PDB ID:6QGH) (Figure 2b), Bax (PDB ID:5W62) (Figure 2c), and beta actin (PDB ID:2BTF) (Figure 2d) and 3D structure of the selected target protein with quinoxaline complex is shown in (Figure 4a-d, Figure 5a (2BTF: β actin), Figure 5b (5b- 4ZAU: EGFR), Figure 5c (5W62: BAX) and Figure 5d (6QGH: Bcl-2). The molecular docking statistics of the quinoxaline and different target proteins complex are represented in Table 1). The protein-ligand complex's native-like structures are identified by docking studies. After the ligand and water molecules that had already formed a connection were removed, the chosen protein complex was employed. Software called Schrodinger/glide was used to do the docking. The following is a statement of the full docking steps. To begin with, water molecules were removed from the protein structures, and the PDB files of 4ZAU, 6QGH, 5W62, and 2BTF were used as input for the software. Kollman charges were assigned to the macromolecules using Schrödinger/Glide, and the structures were examined for any missing atoms. These missing atoms were then repaired, followed by the addition of hydrogen atoms using default parameters. Once all necessary modifications were completed, the updated macromolecules were saved in PDB format within the same directory, and ligand preparation was subsequently initiated. Similar to the macromolecule, Kollman and Gasteiger charges were assigned to the ligand, and torsional flexibility was defined by modifying certain rotatable bonds. The root of the ligand was identified, and adjustments were made to convert rotatable bonds into non-rotatable ones, and vice versa, optimizing the number of active torsions to involve more atoms. The ligand was then saved in the .pdbqt format. Following ligand and macromolecule preparation, rigid residues were generated using the GRID module available within the Schrödinger Glide Grid tool. This program was run using a searching grid extended over ligand molecules with box spacing 101 × 101 × 101; spacing was 0.375A˚ for 4ZAU; 80 × 80 × 80, spacing was 0.375A˚ for 6QGH; 60× 60× 60; spacing was 0.375A˚ for 5W62; 60× 110× 100; spacing was 0.375A˚ for 2BTFwhile other parameters were default. The flexible macromolecule was saved with .pdbqt extension. It employed a conf file referring pdbqt files of macromolecule and compounds prepared using Schrodinger/glide and Grid properties. As an output Schrodinger/glide generated log files and pdbqt files of energy models for the selected data set. The lowest energy model against each ligand was chosen from several energy models in the output file and attached to the end of the original protein file. In order to understand the docking results, interactions between the target protein and the compounds were discovered when the target protein docked with the data set of chemicals. It was hypothesised that each compound's most stable conformation for binding to the protein active site would have the best docking poses. The results of the docking operation were therefore examined using Schrodinger/glide.

Results

Antioxidants assay

The radical scavenging activities of the sample were evaluated at different concentrations (20, 40, 80, 160 and 320 µg/mL). All assays (FRAP, DPPH, H_2O_2 scavenging, ABTS, superoxide, and reducing power) showed a concentration-dependent increase in antioxidant activity, with linear regression curves presented in Tables 2–7 and Figures 1a–1f. According to these findings, the test sample has a concentration-dependent capability of scavenging free radicals that is comparable to that of common antioxidants like ascorbic acid. The IC50 values

Table 1. Molecular Docking Statistics of the Quinoxaline and Different Target Proteins Complex

	EGFR	Bcl2	Bax	β actin
Binding energy (Kcal/mol)	-7.49621	-7.34074	-7.34656	-7.86125
Ligand Efficiency	-0.46	-0.48	-0.37	-0.52
Inhibition Constant	458.18 (mm)	289.76(µm)	1.87 (nm)	150.13 (µm)
Intermolecular Energy (Kcal/mol)	-4.56	-4.83	-3.72	-5.22
vdW + Hbond + desolv Energy (Kcal/mol)	-4.47	-4.82	-3.73	-5.21
Electrostatic Energy	-0.08	0	-0.01	-0.01
Total Internal Energy	0	0	0	0
Torsional Free Energy	0	0	0	0
RMSD	0.01	0.04	0.02	1.48

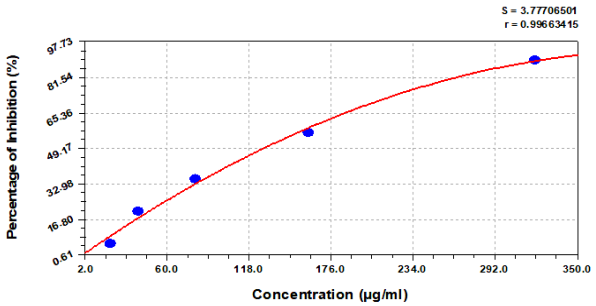


Figure:1a DPPH assay

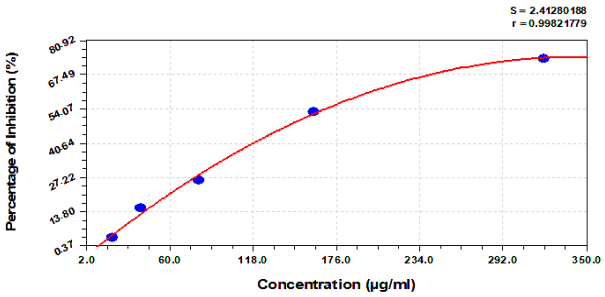


Figure:1b FRAP assay

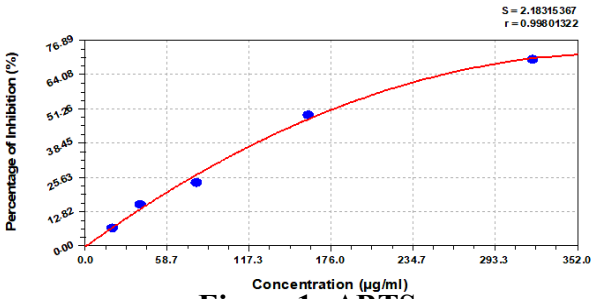


Figure:1c ABTS

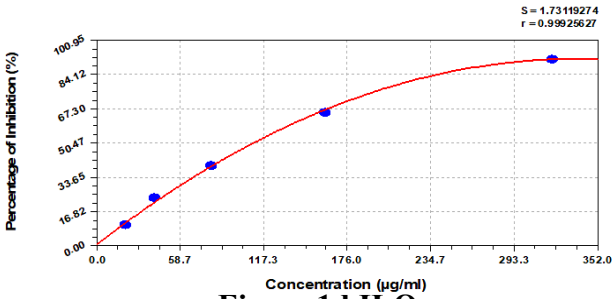


Figure:1d H_2O_2

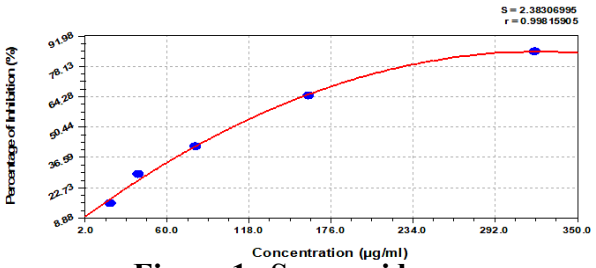


Figure:1e Superoxide assay

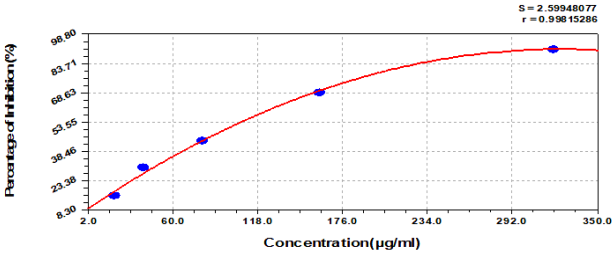


Figure:1f Reducing assay

Figure 1a–1f. DPPH, FRAP, ABTS, H_2O_2, superoxide, and reducing power assays demonstrated a concentration-dependent increase in antioxidant activity. The regression curves for the sample at different concentrations (20, 40, 80, 160, and 320 µg/ml) were linear, indicating that the percentage of inhibition increased proportionally with increasing concentration.

are, for DPPH assay 130.446, FRAP assay 151.343, ABTS 171.551, H_2O_2 108.194, superoxide assay 104.592 and reducing assay 95.893.

Docking study

Table 2. The DPPH Free Radical Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 130.446

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC_{50} Value (µg/ml)
1	20	0.818	0.865	6.127	130.446
2	40	0.707	0.865	20.599	
3	80	0.594	0.865	35.332	
4	160	0.432	0.865	56.453	
5	320	0.18	0.865	89.309	

Table 3. The FRAP Free Radical Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 151.343

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC50 Value (µg/ml)
1	20	0.565	0.587	3.747	151.343
2	40	0.496	0.587	15.502	
3	80	0.433	0.587	26.235	
4	160	0.276	0.587	52.981	
5	320	0.153	0.587	73.935	

Table 4, The ABTS Free Radical Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 171.551

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC50 Value (µg/ml)
1	20	0.725	0.778	6.812	171.551
2	40	0.656	0.778	15.681	
3	80	0.593	0.778	23.778	
4	160	0.396	0.778	49.1	
5	320	0.281	0.778	63.881	

Table 5. The H_2O_2 Free Radical Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 108.194.

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC50 Value (µg/ml)
1	20	0.586	0.656	10.67	108.194
2	40	0.501	0.656	23.628	
3	80	0.396	0.656	39.634	
4	160	0.226	0.656	65.548	
5	320	0.054	0.656	91.768	

Table 6. The Superoxide Free Radical Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 104.592.

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC50 Value (µg/ml)
1	20	0.671	0.797	15.809	104.592
2	40	0.566	0.797	28.983	
3	80	0.361	0.797	54.705	
4	160	0.191	0.797	76.035	
5	320	0.119	0.797	85.069	

Table 7. The The Reducing Assay Scavenging Activity of the Sample Showed Dose Dependent Increased Inhibition and the IC_{50} Value for the Given Sample is 95.893

S.No	Concentration (µg/ml)	Absorbance	Blank	Percentage of Inhibition (%)	IC50 Value (µg/ml)
1	20	0.616	0.732	15.846	95.893
2	40	0.511	0.732	30.191	
3	80	0.409	0.732	44.125	
4	160	0.226	0.732	69.125	
5	320	0.064	0.732	91.256	

The ligand that docks into the active sites of EGFR (4ZAU), Bcl2 (6QGH), Bax (5W62), and beta-actin (2BTF) can be used to explain the binding affinity. This active docking will produce a potential binding energy score. In this case, stronger and more stable ligand-protein interactions are explained by more negative or lower binding energy values. EGFR is a transmembrane tyrosine kinase receptor that promotes cell survival and proliferation and is commonly overexpressed in a variety of malignancies. According to the results of molecular docking, quinoxaline has a strong affinity for the EGFR active site, indicating that it may have an EGFR inhibitory effect. Such inhibition may hinder the proliferation of cancer cells by interfering with downstream signalling pathways, such as the MAPK and PI3K/AKT pathways. Quinoxaline's binding to Bcl-2, an anti-apoptotic protein that is frequently over expressed in cancers, results in treatment resistance. According to docking findings, quinoxaline and Bcl-2 have persistent contacts that may counteract the protein's activity and favour apoptotic induction. Quinoxaline may improve the apoptotic cascade by either directly activating Bax or indirectly modulating it through Bcl-2 inhibition, as Bax, a pro-apoptotic homologue, and also shown beneficial interaction patterns. In order to evaluate any non-specific interactions or possible off-target effects, β-actin, a structural cytoskeletal protein implicated in cell movement and metastasis was also included in the analysis. Quinoxaline's binding with the main cancer targets was more substantial than its interaction profile with β-actin, suggesting a positive selectivity profile. The Lipinski's 'rule of five' is the first qualitative attempt to guide the design was used to understand the molecular weight and number of hydrogen-bond donors and acceptors (Table 8). The quinoxaline

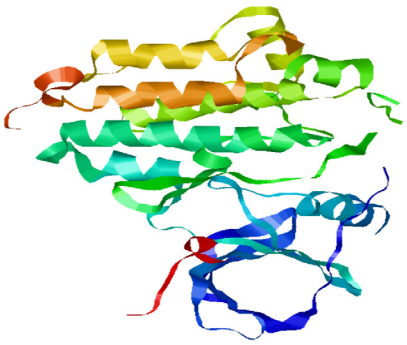


Figure 2a EGFR (4ZAU)

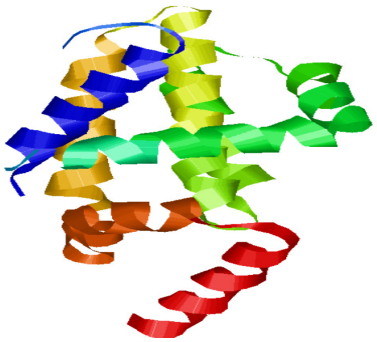


Figure 2b Bcl2 (6QGH)

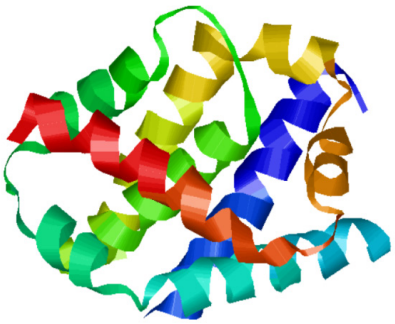


Figure 2c Bax (5W62)

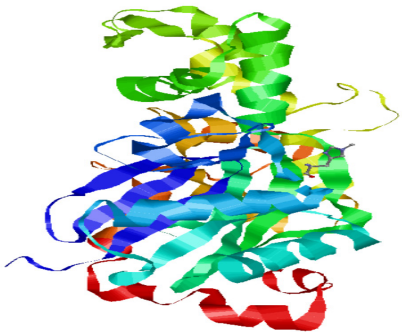
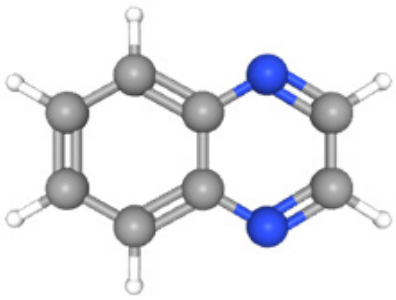


Figure 2d Beta-actin (2BTF)

Figure 2a-2d. 3D Structure of Target Proteins without Ligands (2a) EGFR (4ZAU), (2b) Bcl2 (6QGH) (2c) Bax (5W62) and (2d) Beta-actin (2BTF)



Quinoxaline

Figure 3. Chemical Structure of Quinoxaline

exhibited a stronger affinity toward cancer-related targets EGFR and Bcl-2, than toward β-actin, indicating target selectivity. This difference can be attributed to the structural compatibility of quinoxaline with the active or regulatory binding sites of EGFR, Bcl-2, and Bax, whereas β-actin lacks such well-defined ligand-binding pockets. Thus, the weaker interaction with β-actin reflects a favorable selectivity profile, supporting quinoxaline's potential as a targeted anticancer agent. Furthermore the potential nature of the quinoxaline as multi-target medicines in cancer therapy, able to suppress proliferative signalling and induce apoptosis, is generally supported by these in-silico results. Molecular Docking statistics of the quinoxaline and different target proteins complex (Table 1) explains β-actin had a slightly higher RMSD but the strongest binding energy and ligand efficiency. Bax was the most sensitive to quinoxaline binding, as evidenced by its lowest inhibition constant (1.87 nM).

Discussion

Oncological research has evolved from the use of broadly cytotoxic agents to the development of safer and more selective therapeutics that precisely target malignant cells. In the pursuit of alternative treatment strategies, natural compounds have gained significant attention, particularly those exhibiting potent antioxidant properties, which may contribute to cancer prevention and therapy. These antioxidants scavenge the free radicals which cause DNA damage and further lead to the development of the cancer. In our current investigation, we evaluated the in vitro assays like DPPH, hydrogen peroxide scavenging, FRAP, ABTS, superoxide radical scavenging, and reducing power assays. For all the antioxidant assays,

Table 8. Compounds (Lipinski Rule of Five) Details Taken from SwissADME

Compound	PubChem CID	Molecular formula	Molecular weight (g/mol)	Hydrogen bond donor	Hydrogen bond acceptor
Quinoxaline	7045	C8H6N2	130.15	0	2

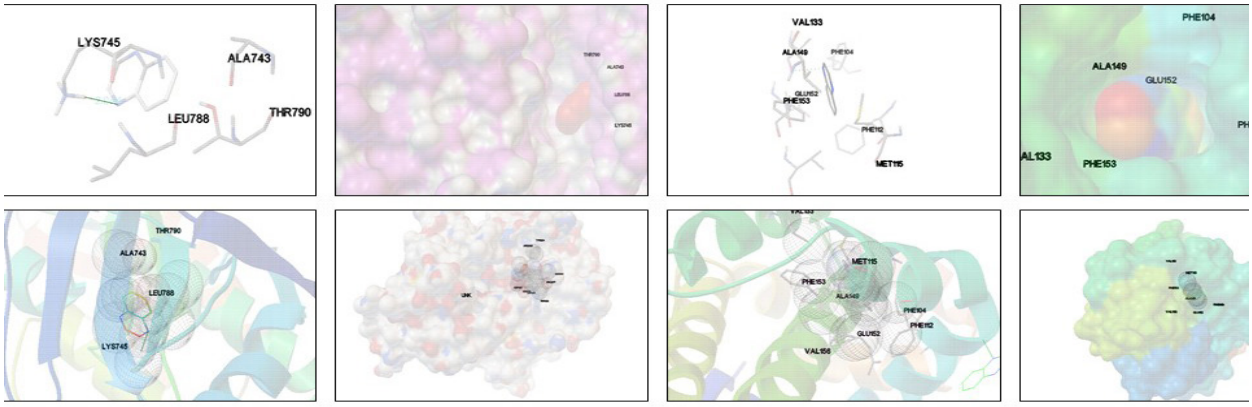


Figure 4a- EGFR

Figure 4b- Bcl2

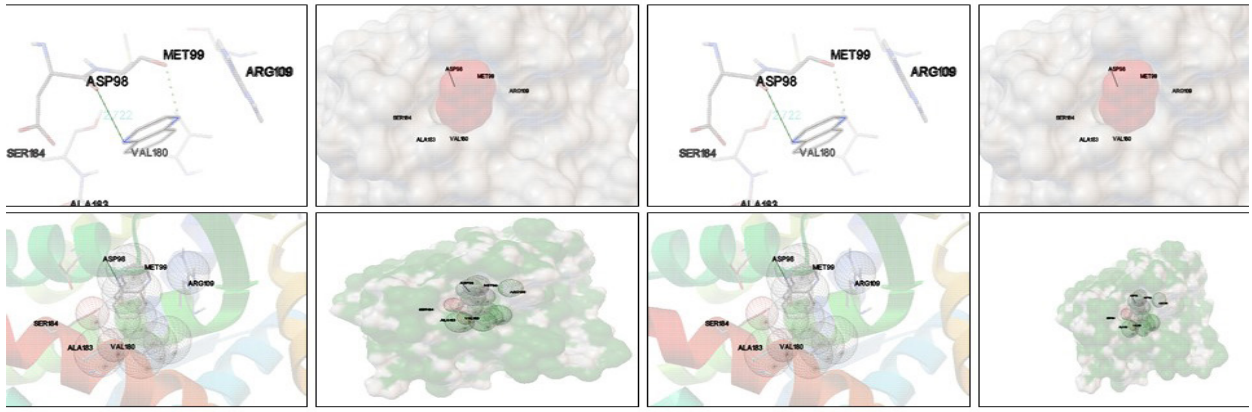


Figure 4c- Bax

Figure 4d- Beta actin

Figure 4a-4d. 3D Structure of Selected Target Proteins with Quinoxaline Complex

our sample showed concentration based antioxidant activity. As the concentration increases the inhibition also increased. The IC_{50} values for the antioxidant assays are 130.446, 151.343, 171.551, 108.194, 104.592, and 95.893. These results suggest that the sample have potential scavenging nature against free radicals. In all the assays the standards also used to study the comparative standards for the sample. The primary drawbacks of chemotherapeutic agents are their lack of selectivity and the resulting toxicity. Therefore, the enhancement of targeted therapy with site-specific action in the management of this deadly illness is well supported by evidence. Quinazoline pharmacophore-based derivatives have been extensively explored as a kinase-targeted cancer therapy as a result of extensive work in the drug discovery area and knowledge of cancer biology. The FDA has approved a number of quinazoline-based medications that function as kinase inhibitors and are now clinically used to treat advanced cancers. In this context, the present study investigates the molecular docking interactions of quinoxaline a heterocyclic scaffold known for its diverse pharmacological properties with a panel of cancer-associated proteins including EGFR, Bcl-2, Bax and β-actin. Given that cancer cells' resistance to apoptosis is largely due to anti-apoptotic Bcl-2 proteins, certain medications that block these proteins [31]. Due to its critical role in regulating cell survival, proliferation, metastasis, and angiogenesis, and its frequent overexpression in numerous solid tumors, the (EGFR) is considered a promising therapeutic target for cancer treatment [32, 33]. The molecular docking analysis revealed the binding affinity of the ligand to EGFR, as indicated by binding energy values and specific interaction patterns within the protein's active site. The quinoxaline and different target proteins Complex's molecular docking statistics show that β-actin exhibited the strongest binding energy and ligand efficiency, while having a slightly greater RMSD. Bax's lowest inhibition constant (1.87 nM) indicated that it was the most susceptible to quinoxaline binding.

Quinoxaline's strong interactions with EGFR and Bcl2 make it a multi-target potential anticancer medication. Bcl-2 protein inhibitors have limited effect on normal cells because tumor cells express the protein at significantly higher levels than do normal cells. Based on tumor pathophysiology, a unique treatment approach involves blocking the Bcl-2 anti-apoptotic protein to overcome tumor cells' resistance to apoptosis [34]. The findings of research on actin expression in invasive and non-invasive cancer cells deserve special attention. Comparing invasive and non-invasive sarcoma cell lines, it was discovered that the former had significantly larger levels of β-actin. It was also accompanied by modifications in the concentration and subcellular distribution of β-actin at the apical regions of the developing pseudopods, or regions with high actin polymerisation dynamics. Our findings on the identification

Figure 5a- 2BTF: β actin
Best Ligand Pose: energy = -7.86125 kcal/mol
Docking run: elapsed time = 46 seconds

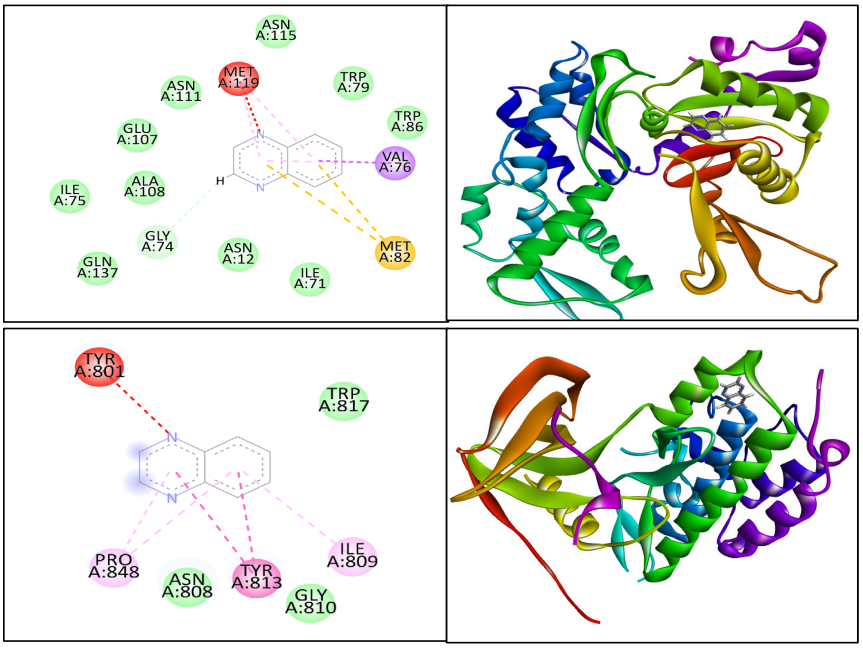


Figure 5b- 4ZAU: EGFR
Best Ligand Pose: energy = -7.49621 kcal/mol
Docking run: elapsed time = 35 seconds

Figure 5c- 5W62: BAX
Best Ligand Pose: energy = -7.34656 kcal/mol
Docking run: elapsed time = 15 seconds

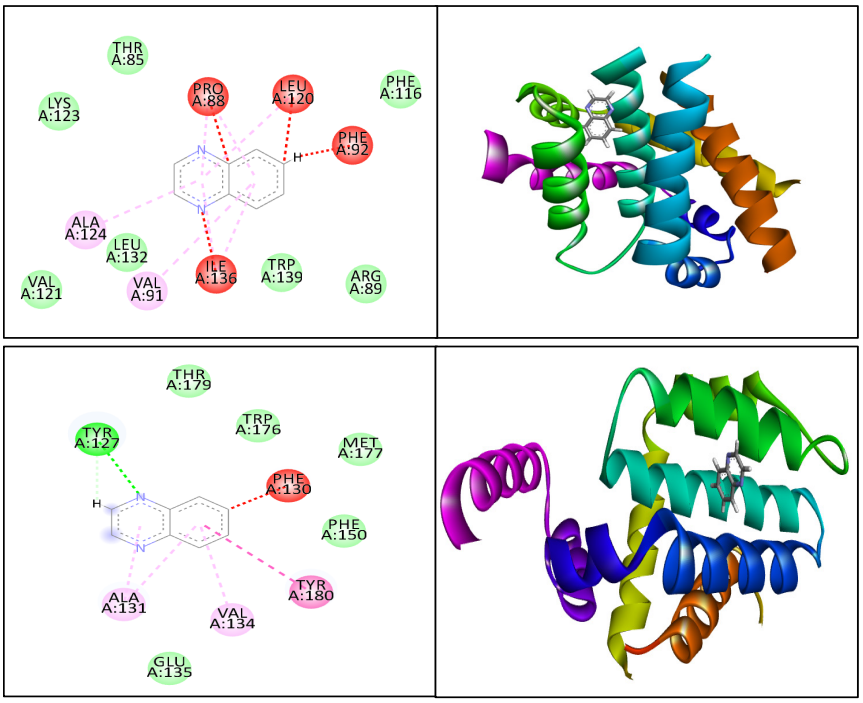


Figure 5d- 6QGH: Bcl-2
Best Ligand Pose: energy = -7.34074 kcal/mol
Docking run: elapsed time = 19 seconds

Figure 5a-5d. Molecular Docking Statistics of the Quinoxaline and Different Target Proteins Complex.

of β-actin using immunofluorescent staining in human adenocarcinoma cell types, which are distinguished by their varying capacity for metastasis, appear to be the most intriguing [34, 35]. This study also discovered that quinoxaline's binding to Bcl-2, an anti-apoptotic protein that is overexpressed in cancers, indirectly led to treatment resistance. According to docking findings, quinoxaline and Bcl-2 have persistent contacts that may counteract the protein's activity and favour apoptotic induction. When combined with molecular docking and antioxidant results, the interactions of quinoxaline with Bax and Bcl-2 provide a clearer understanding of its role in regulating apoptosis and combating oxidative stress. We can confidently state that quinoxaline has the ability to combat cancer and can be utilised to treat any condition linked to oxidative stress after taking all of these factors into account. Quinoxaline may improve the apoptotic cascade by either directly activating Bax or indirectly modulating it through Bcl-2 inhibition, as Bax, a pro-apoptotic homologue, and also shown beneficial interaction patterns. Additionally, β-actin, a structural cytoskeletal protein implicated in metastasis and cell motility, was incorporated to evaluate any possible off-target effects or non-specific interactions. Compared to its binding with the principal cancer targets, quinoxaline's interaction profile with β-actin was less significant, suggesting a positive selectivity profile. By specifically targeting important apoptotic regulators, quinoxaline has shown promise in promoting apoptosis. The results of antioxidant and molecular docking studies indicate a dual method of apoptosis induction, with strong contacts with anti-apoptotic bcl-2 that may restrict its activity and favourable binding with pro-apoptotic bax.

Author Contribution Statement

Cecileya Jasmin Meshak Dhanashekaran: Conceptualization, Project administration, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. Vinod Prabhu Venugopal: Data curation, Formal analysis, Investigation, Methodology. Nivetha Chokkalingam and Sakthivel Kunnathur Murugesan: Data curation, Formal analysis, Investigation, Methodology. Radhakrishnan Narayanaswamy: Data curation and Formal analysis.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability

Data's are available upon request.

Declaration

The study is not registered in any registration dataset (for clinical trials, guidelines and meta-analysis).

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