SYNTHESIS, CHARACTERIZATION, DOCKING AND STUDY OF QUINAZOLINES AND THEIR ANTI-CANCER ACTIVITY AGAINST BREAST CARCINOMA

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ABSTRACT: Quinazolines is a versatile heterocyclic compound with diverse pharmacological properties. In this study, we conducted a comprehensive analysis combining IR spectroscopy, NMR spectroscopy, Mass spectroscopy, and molecular docking to investigate the structure, properties, and potential drug-receptor interactions of quinazoline derivatives. In the present study quinazoline derivatives associated with quinazoline ring were synthesized in moderate to good yield by conventional method. Efficient, multi-component synthesis of 2-(4-Chlorostyryl)-4-hydrazinyl quinazoline derivatives in excellent yields, at room temperature using ethanol as a solvent. Docking analysis against DNA topoisomerase 2β, vascular endothelial growth factor receptor 2 and B-DNA. The compounds showed good to moderate bioactivity score against several human receptors. Over all anticancer activity of synthesized compounds is moderate as compared to standard drug but when higher concentration was used the activity looks to be strong. Compounds containing quinazoline ring has higher activity which clearly indicates enhanced anticancer activity. According to IC50 value compound 5 is more potent than compound 4 because value of IC50 compound 5 is 224 and value of IC50 compound 4 is 1368 it means compound having low IC50 value shows higher anticancer activity.

Keywords: Quinazoline, Mass Spectroscopy, Molecular Docking, Anticancer-activity.

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INTRODUCTION:

Quinazoline (1, 3-diazanaphthalene; 1) is a moiety made up of two condensed six-membered aromatic rings, a pyrimidine ring, and a benzene ring. It is yellow and amorphous, and its molar mass is 130.15 g. mol^{-1}, and the chemical formula is $C_8H_6N_2$ (1). Anticancer drug**,** also called anti-neoplastic drug**,** any drug that is effective in the treatment of malignant,

or cancerous, disease Cancer is a very complex group of diseases characterized by the uncontrolled abnormal cell growth and spread of abnormal cells in the body. There are many types of cancer, each with its own specific characteristics and treatment options. Anti-cancer drugs are medications designed to target and inhibit the growth of cancer cells or destroy them. These drugs can be used alone or in combination with other treatments like surgery, radiation therapy, and immunotherapy, depending on the type and stage of cancer. There are several major classes of anticancer drugs; these include alkyl ting agents, anti-metabolites, natural products, and hormones. The most effective class of anti-cancer drugs that contains the quinazoline chemical structure is known as EGFR (Epidermal Growth Factor Receptor) inhibitors. These drugs target the EGFR pathway, which is often overactive in some types of cancer, like non-small cell lung cancer. Erlotinib and Gefitinib are examples of EGFR inhibitors that contain a quinazoline moiety in their chemical structure. It's important to note that cancer treatment is highly individualized based on the type of cancer, its stage, and the patient's overall health. Different drugs and therapies are used for different types of cancer, and new treatments are continually being developed as our understanding of cancer biology advances It's important to note that the choice of anti-cancer drug depends on the type and stage of cancer, as well as the patient's individual characteristics and medical history. Cancer treatment often involves a combination of therapies, including surgery, radiation therapy, immunotherapy, and targeted therapy in addition to chemotherapy (2). Molecular docking is a computational technique widely used in the pharmaceutical field to predict the interactions between small molecules (ligands) and target proteins (receptors). It plays a crucial role in drug discovery and design by helping researchers understand how potential drug compounds may bind to their target proteins and influence their biological activity (3). Here are the key aspects of molecular docking in the pharmaceutical field-

Target Identification: Before molecular docking can be performed, researchers must identify a specific protein target that is associated with a disease or condition. This target is often a receptor, enzyme, or other biomolecule involved in the disease's biological pathway.

Ligand Preparation: Ligands are the small molecules or drug candidates that researchers want to study. These ligands are prepared by optimizing their three-dimensional (3D) structures, ensuring they are in a suitable format for docking studies.

Protein Structure: The 3D structure of the target protein is crucial for molecular docking. Experimental techniques like X-ray crystallography or NMR spectroscopy can provide this

structural information. If the experimental structure is not available, homology modeling or other computational methods can be used to predict the protein's structure.

Docking Algorithms: There are various molecular docking software tools and algorithms available, such as AutoDock, AutoDock Vina, GOLD, and Glide. These programs use different scoring functions and search algorithms to predict the energetically favorable binding poses and binding affinities of ligands within the protein's binding site.

Scoring Functions: Docking software relies on scoring functions to estimate the binding affinity of a ligand to the target protein. These functions consider various factors, including van der Waals forces, electrostatic interactions, hydrogen bonding, and solvation energy. The ligand poses with the lowest energy scores are considered the most likely binding conformations.

Virtual Screening: Molecular docking can be used for virtual screening, where a large database of potential ligands is screened to identify compounds with high binding affinities for the target protein. This helps in identifying potential drug candidates efficiently.

Binding Site Analysis: Docking can also provide insights into the binding site's characteristics, such as key amino acid residues and the types of interactions (e.g., hydrogen bonds, hydrophobic interactions) that stabilize the ligand-protein complex. This information is valuable for rational drug design.

Lead Optimization: Once promising ligands are identified through docking studies, medicinal chemists can use this information to design and synthesize analogs with improved binding affinities and pharmacological properties.

Experimental Validation: The results of docking studies are typically validated through experimental techniques like binding assays, isothermal titration calorimetry (ITC), or surface plasmon resonance (SPR) to confirm the predicted binding affinity and mode of interaction.

Docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands). The Glide XP docking procedure and the Schrodinger Maestro GUI were used to docking.

Molecular docking is a valuable tool in the early stages of drug discovery, helping to narrow down the pool of potential drug candidates, optimize lead compounds, and understand the molecular basis of drug-protein interactions. However, it is important to note that docking

results are computational predictions and should be followed up with rigorous experimental testing to confirm their accuracy and applicability in drug development (4).

METHODS:

All chemicals used were of Laboratory Reagent (LR) Grade. The synthesized compounds were characterized by melting point, TLC, IR, MS and NMR. Thin layer chromatography was performed using Silica Gel G coated on glass plates and the spots were visualized by expo sure to iodine. (5)

1. **2-methylquinazolin-4(3H)-one**

Heat a solution of Anthranilic acid (0.01mol) in acetic anhydride (2.5%) under refluxfor10minutes at 210 watt. Add ammonium acetate (5.0%) to the same in situ. Cool the solution, filter it & wash with water. Finally recrystalised from ethanol (6ml/gm). Percentage Yield- 62% , m.p. 239-241^{0}C.

2. **4-Chloro-2-(4-chlorostyryl) quinazoline**

2-(4-Chlorostyryl) quinazolin-4(3H)-one (3.5 g, 0.123 mol) was refluxed in excess phosphorous oxychloride (5mL) for 14 h and in presence of a catalytic amount of tri ethylamine (3 - 5 drops). The reaction mixture was allowed to cool then poured onto crushed ice. The obtained solid was collected. Yellowish brown, yield%:82%, m.p.:268˚C-270˚C (6).

3. **2-(4-Chlorostyryl)-4-hydrazinylquinazoline;**

A mixture of 2-(4-Chlorostyryl)-4-hydrazinylquinazoline(3.2 g, 1mol) and hydrazine hydrate 99% (0.5 g, 0.5mL, 5 mol) (1:5) was refluxed in absolute ethanol (30mL) for 8 h. The reaction mixture was allowed to cool and poured on crushed ice. The obtained solid was filtered, dried and recrystallized from acetone/ethanol. Yellow crystals, yield%: 42%, m.p. 180˚C -182˚C (7).

4. **2-(4-Chlorostyryl)-4-hydrazinylquinazoline**

A mixture of compound **3**(3.2 g, 1mol) and hydrazine hydrate 99% (0.5 g, 0.5mL, 5 mol) (1:5) was refluxed in absolute ethanol (30mL) for 8 h. The reaction mixture was allowed to cool and poured on crushed ice. The obtained solid was filtered, dried and recrystallized from acetone/ethanol (8).

5. 2-[(4-chlorostyrl)-4-hydrazinylquinazoline]methyl-9H-pyrido[3,4-6]indol-3 carboxylate

A mixture of compound **3** (3 g, 1mol) and compound **4** (3 g, 1mol) was refluxed in absolute ethanol (20ml) for 14 hr. The reaction mixture was allowed to cool and poured on crushed ice. The obtained solid was filtered, dried and recrystallized from acetone/ethanol (9).

DOCKING:

Molecular docking simulation gave an insight into favorable docking sites, of the compounds within DNA grooves (having 4 Å surrounding area), and closeness and snugness of fit of the docked ligands (5a-h) with DNA via H bonding, hydrophobic and VDW interactions. It was found that the DNA minor groove was the most appropriate site for the binding of the compounds as is apparent from the obtained BE and K d values Compound 5g displayed the highest affinity towards B-DNA (3, 10).

RESULT AND DISCUSSION:

The research work was aimed to synthesize some novel Quinazoline derivatives associated quinazoline with ring which has different potent pharmacophores and to explore their anticancer activities.

PHYSICAL AND SPECTRAL CHARACTERISTICS

Physical characteristics:

All the synthesized compounds were light cream to white colored crystalline solids compounds are freely soluble in chloroform and other solvents like methanol, ethanol. The melting points of the compounds were in the range of 231ºC to 233ºC.

IR spectra of all compounds were recorded on FT-IR8400S Shimadzu Spectrophotometer using KBr disc. All the synthesized compounds have shown characteristics trenching and bending in desired range.

Mass spectra

Mass spectra were obtained using Agilent 6520(Q-TOF). All the spectra were taken by direct infusion mass with ESI and APCI in positive and negative mode ionization ranging from 50-500m/e. All the compounds possess a molecular ion M^+ peak and base

peak.

^{1}HNMR spectra

The ^{1}H NMR spectra of some of the compounds were studied in $CDCl_3$ on a SPECT 400MHz NMR spectrometer. All the compounds show characteristic chemical shift from TMS in terms of δ ppm. δ value obtained in the desired range which signifies the presence of aromatic ring.

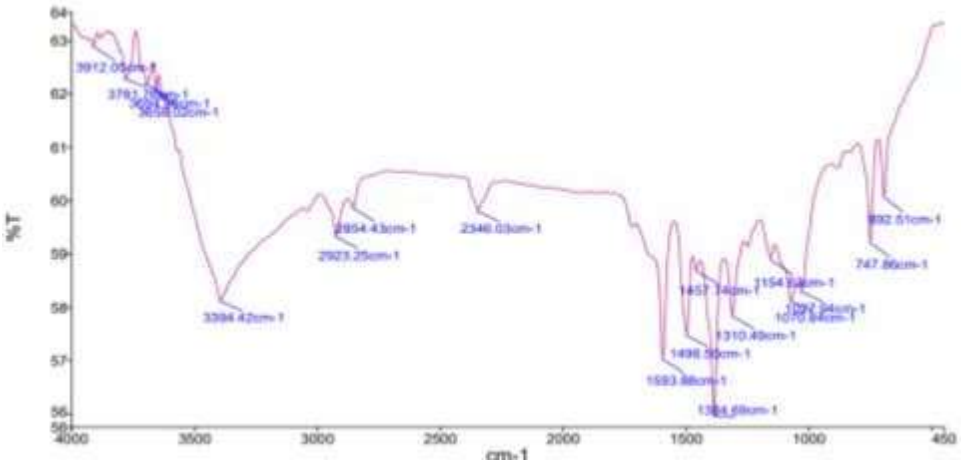


Figure 1: IR spectra of compound 4

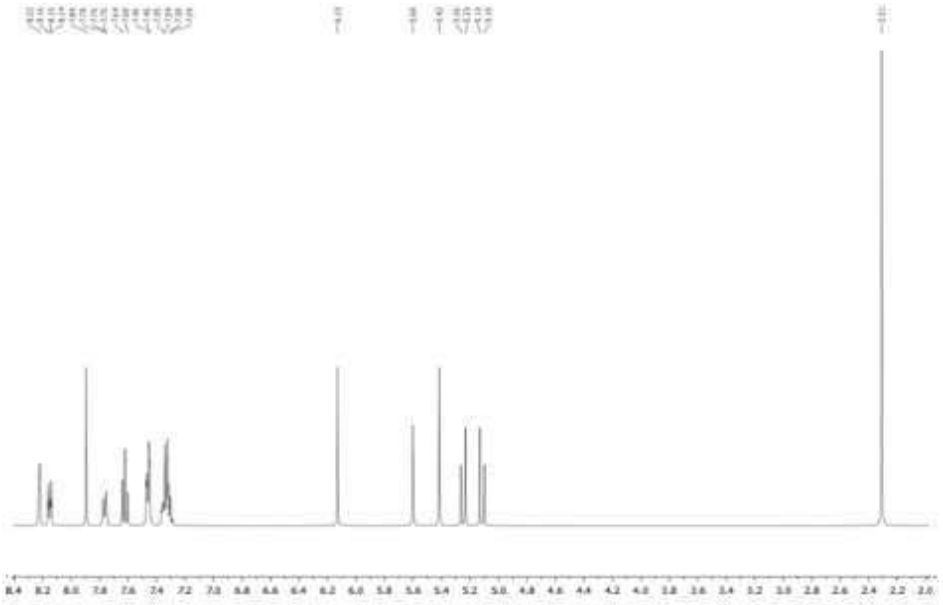


Figure 2: ^{1}HNMR spectrum of Compound4

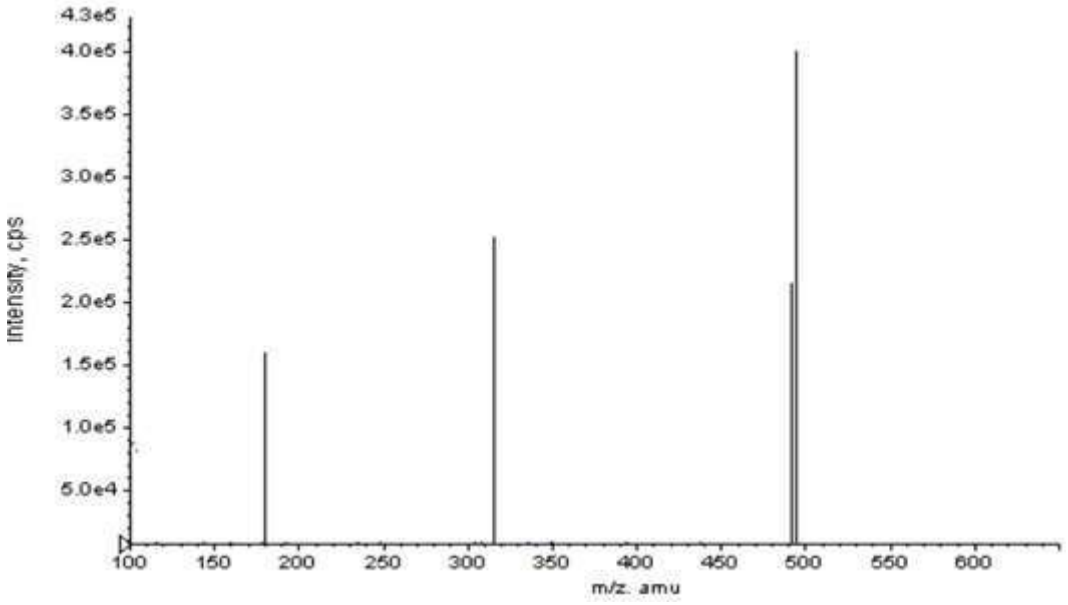


Figure 3: Mass Spectrum of compound 4

pound No.	**IR(cm^{-1})**	**^{1}HNMR(δ)**	**Mass(m/z)**
4(a)	3694,3658(O-H str.), 3394,2923(N-H str.), 2854(C-H str.), 2346(O=C=O str.), 1593(C=C str.), 1496,1457(C-H bending.), 1310(S-O str.), 1154(C=O str.), 1070(S=O str.), 747(C-H bending.), 592(C-I str.)	9.33(s,1H,-NH) 7.77(s,1H,-NH) 7.61(m,4H, Ar-H) 5.39(s,1H,-CH-) 4.89(s,1H,-CH), 2.29(s,3H,-CH_3	495.07$[M+1]^+$ 494.06$[M]^{+\cdot}$ 316.04 [M-C10H8ClN]$^{+\cdot}$ 178.03 [M-C9H8NOS]$^{+\cdot}$

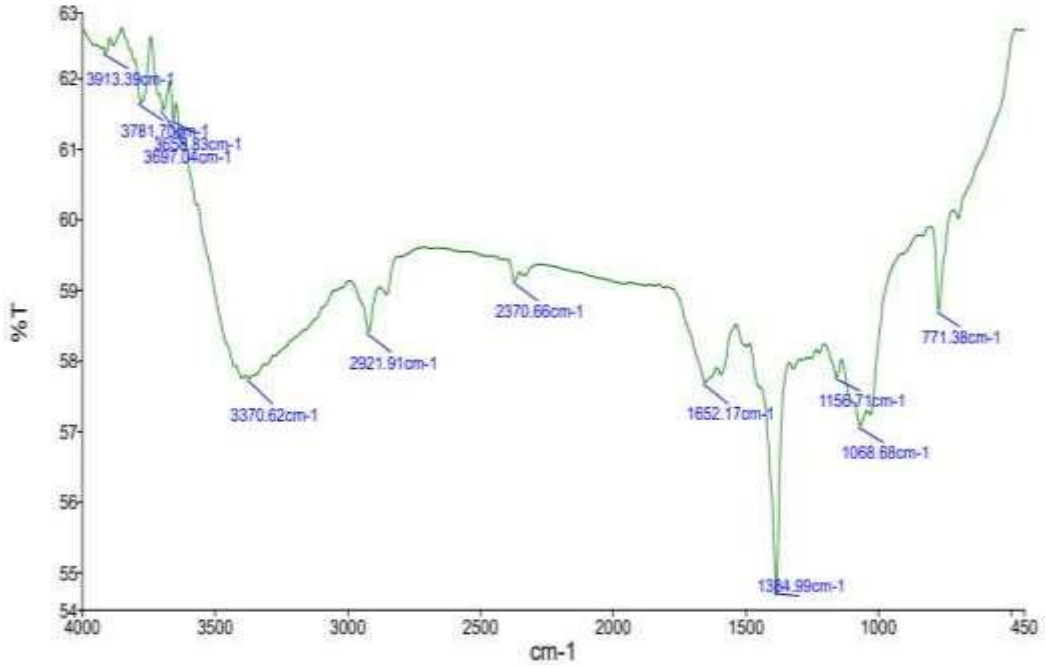


Figure 4: IR spectra of compound 5(a)

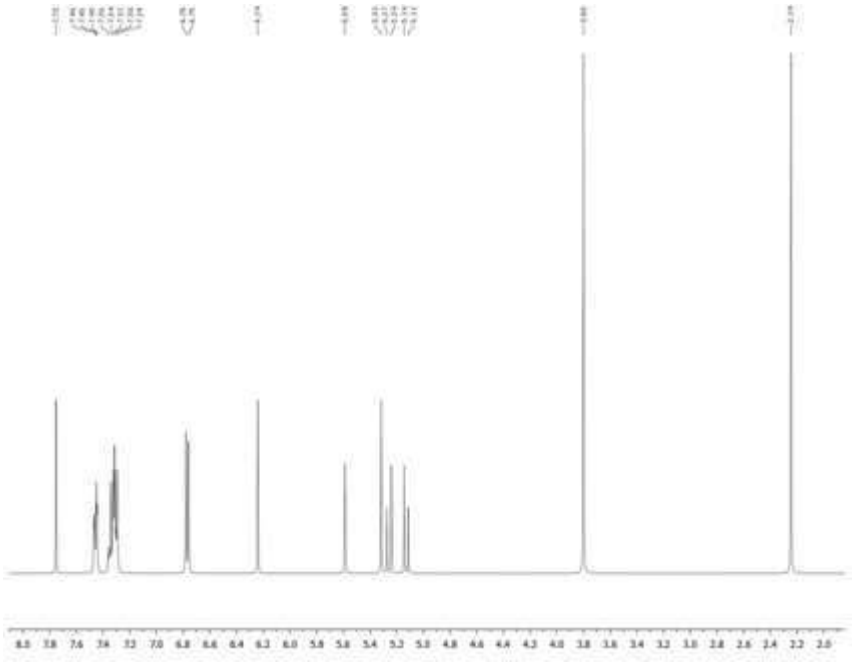


Figure 5: ^{1}HNMR spectrum of Compound 5(a)

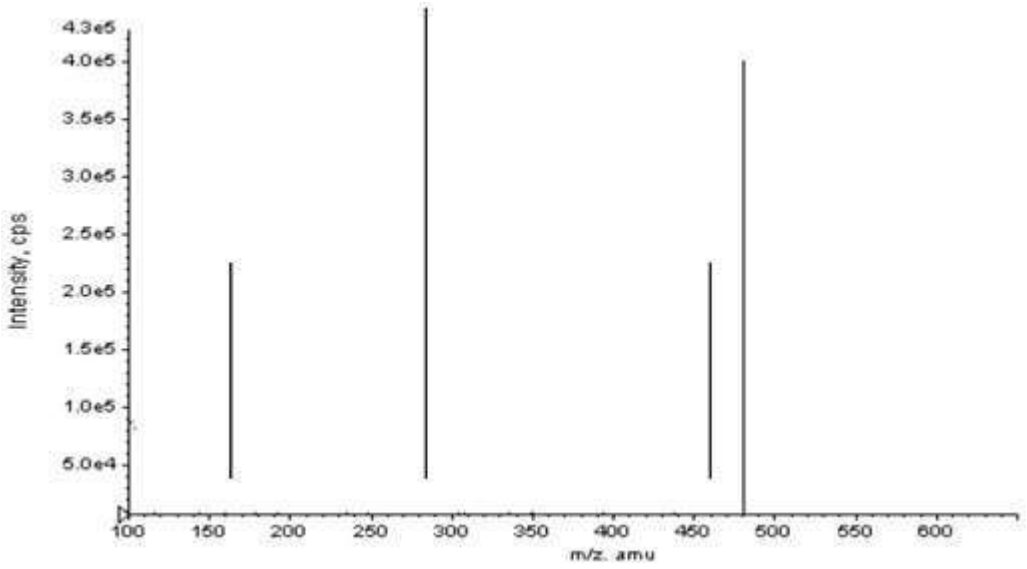


Figure 6: Mass Spectrum of compound 5(a)

Compound No.	IR(cm^{-1})	^{1}HNMR(δ)	Mass(m/z)
5(a)	3697,3658(O-H str.), 3370(N-H str.), 2921(C-H str.), 2370(O=C=O str.), 1652(C=O str.), 1384(C-H bending), 1156(C-O str.), 1068(S=O str.), 771(C-H bending.)	9.10(s, 1H, -NH) 7.63(s, 1H, -NH) 6.85(m, 4H, Ar-H) 5.19(s, 1H, -CH-) 4.75(s, 1H,-CH) 3.71 (s, 3H, $-CH_3$) 2.25 (s, 3H, $-CH_3$)	480.10 $[M+1]^+$479.09[M] +· 301.07 [M-C23H18ClN4O2] 178.03 [M-C9H8NOS]+·

Anti-Cancer activity

Experimental Details

NRU Assay

Cytotoxicity of the provided samples on MCF-7 cell line (Procured from NCCS Pune) was determined by NRU (Neutral Red Uptake) Assay. The cells (5000-8000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-

AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO_2. Next day, medium was removed and fresh culture medium was added to each well of the plate. 5 µl of Treatment dilutions (of different concentrations) were added to the defined wells and treated plates were incubated for 24 h. 100 µl of NRU (SRL Chem-36248) (40 µg/ml in PBS - phosphate buffered saline) was added to the defined wells and incubated (Heal Force-Smart cell CO_2 Incubator-Hf-90) for 1 h. After that medium was removed, NRU was dissolved in 100 µl of NRU Desta in solution. Finally plates were read at 550/660 nm using Elisa Plate Reader (iMark BioRad-USA). IC-50 was calculated by using software Graph Pad Prism -6.

Results

Sample code	IC50 value (µM)
4	1368
5	224

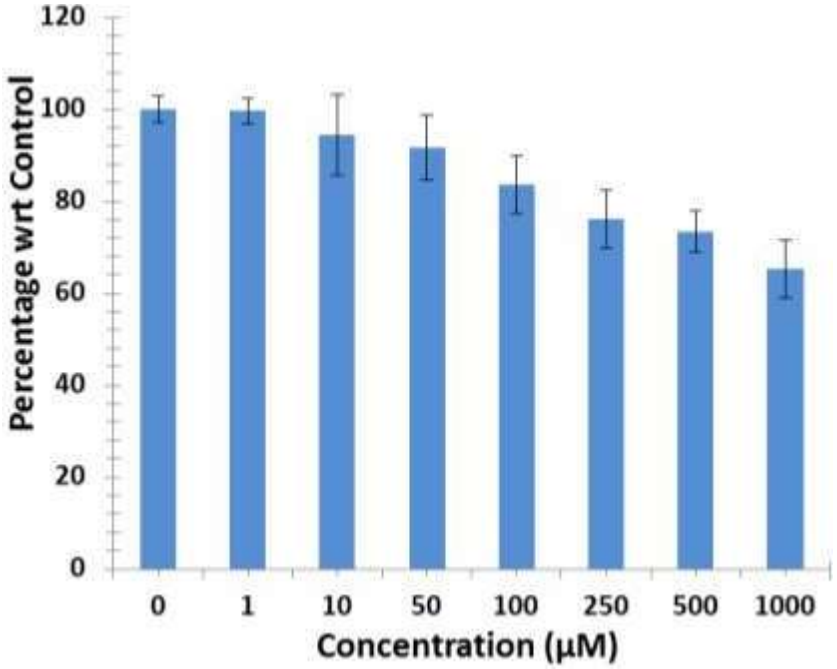


Figure 7: NRU Assay-MCF-7 (4)

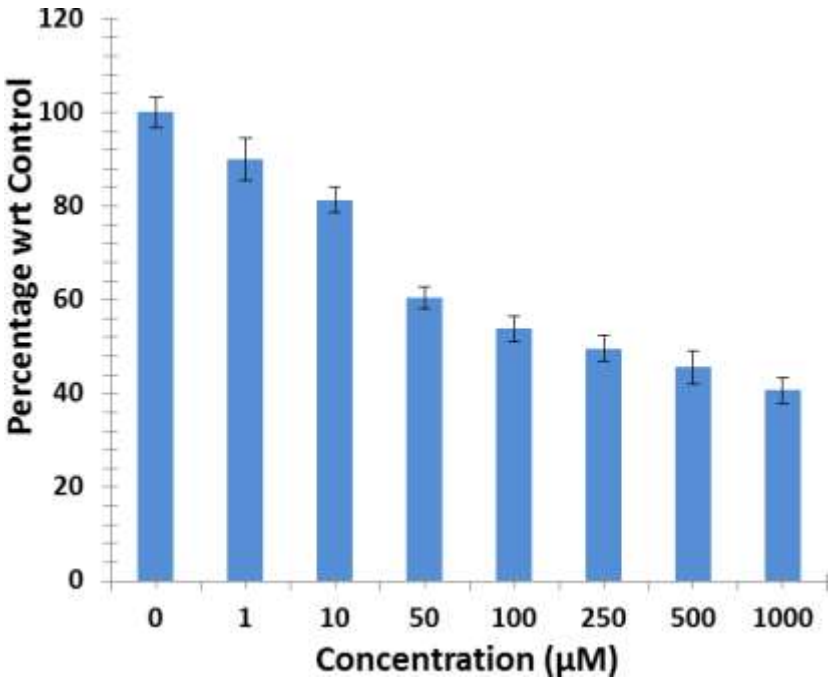


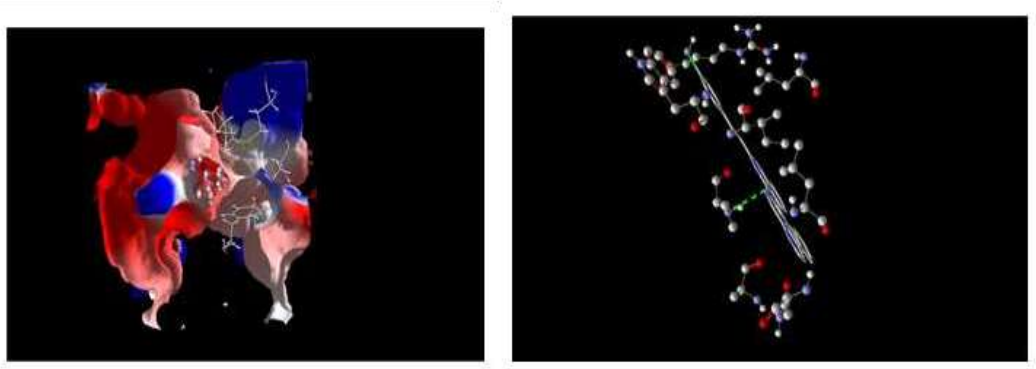
Figure 8: NRU Assay-MCF-7 (5)

Structural characteristics:

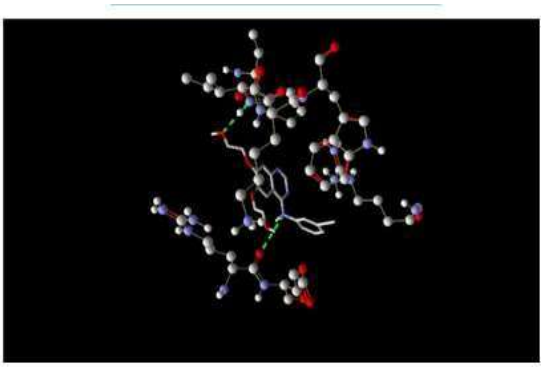
Docking:

Docking analysis was performed using The synthesized 2-(4-Chlorostyryl)-4-hyazinylquinazoline derivatives were subjected to molecular docking evaluations using suite in order to understand the drug-receptor interaction, prospective binding conformation as well as binding energies and dissociation constant. The docking results were evaluated in the form of BE, Kd, hydrogen bonding and hydrophobic interactions.

Figure: 9 (a) Docking Structure of Compound-3, (b) Docking Structure of Compound-4



(c) Docking Structure of Market Drug



CONCLUSION:

Briefly restate the purpose of the experiment Following tentative conclusions can be drawn from observed data, In the present study quinazoline derivatives associated with quinazoline ring were synthesized in moderate to good yield by conventional method. Efficient, multi-component synthesis of 2-(4-Chlorostyryl)-4-hydrazinyl quinazoline derivatives in excellent yields, at room temperature using ethanol as a solvent. Docking analysis against DNA topoisomerase 2β, vascular endothelial growth factor receptor 2 and B-DNA. The compounds showed good to moderate bioactivity score against several human receptors. Over all anticancer activity of synthesized compounds is moderate as compared to standard drug but when higher concentration was used the activity looks to be strong. Compounds containing quinazoline ring has higher activity which clearly indicates enhanced anticancer activity. According to IC50 value compound 5 is more potent than compound 4 because value of IC50 compound 5 is 224 and value of IC50 compound 4 is 1368 it means compound having low IC50 value shows higher anticancer activity.

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