Journal of Young Pharmacists, 2024; 16(4):725-734.
https://www.jyoungpharm.org

Original Article

Synthesis, Molecular Docking, *in silico* Druglikeness: *In vitro* Cytotoxicity Study on MCF-7 Cell Line of Quinazolin-4-one Scaffold

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ABSTRACT

Background: Quinazolinone compounds are a preferred class of multi-agent therapeutics in the domains of biology and pharmacology. One of this scaffold's most notable biological functions is its anticancer properties. Numerous well-known quinazolines with anticancer properties operate on diverse molecular targets *via* distinct methods. Hence, the present study is planned to design the new series of substituted Quinazolinone derivatives and synthesize, carry out molecular docking studies for the proposed compounds against EGFR TK-PDB: 1M17 and CDK-PDB: 2KW6 receptor by using PyRx virtual screening tools Auto-dock vina software 0.9 version and to screen the compounds for their *in vitro* cytotoxicity against MCF-7 breast cancer cell line. **Materials and Methods:** A unique set of compounds, dihydro-quinazolinone QS1-QS4, and its scaffold, were created. Recrystallization by ethanol purifies every produced chemical. The protein was downloaded from PDB and Auto-dock vina PyRx (0.8) was used to predict it in silico studies. **Results:** TLC and IR are used to describe each derivative. The produced compounds were tested for their ability to inhibit cancer *in vitro* using the MCF7 cancer cell line and reference medication doxorubicin. The results of the molecular docking study indicated that the synthesized compounds had a good binding affinity for the 1M17 and 2KW6 macromolecules. **Conclusion:** The MTT assay was used to screen the synthesized compounds based on docking score and assess their anticancer activities *in vitro*. The molecule that was produced and evaluated showed increased activity and was also screened for *in vitro* cytotoxicity against the MCF-7 breast cancer cell line, which results in concentration rises and decreases in cell viability.

Keywords: Quinazolin-4-one, MTT assay, MCF-7, Breast cancer cell line, Cytotoxicity, EGFR.

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Received: 19-06-2024;
Revised: 21-07-2024;
Accepted: 11-09-2024.

INTRODUCTION

The pharmaceutical industry views the drug discovery process as a significant matter due to its cost-effectiveness and time-consuming nature, which yields novel medication candidates and expands the range of ailments that can be discovered. The initial step in the drug development process, drug target identification, is taking too long. This often yields ineffective outcomes since traditional methods, such as *in vitro* and *in vivo* are unable to analyze large amounts of data. Pharmaceutical companies now have a great possibility to find new prospective therapeutic targets thanks to sophisticated in silico techniques, which also affects the duration and success of conducting clinical trials to find new targets. This work's primary objective is to investigate *silico* techniques for the drug development process, with a focus on target identification. Over the past ten years, cancer has emerged as one of the deadliest illnesses, wreaking havoc on people worldwide.[1,2] Uncontrolled or aberrant cell proliferation in the body is a severe health concern that caused over 10 million deaths globally in 2020.[3,4] There is a growing need to create lead candidates with fewer side effects and greater potency because of the severe side effects of chemotherapy and the development of treatment resistance brought on by genetic target mutations.[5-7] In malignant situations, a variety of growth factors that may be connected to some abnormalities promote cell proliferation.[8] Signals are transferred from the external domain to the interior of the cell by a variety of growth factors found on the cell surface, including Human Epidermal growth factor Receptor 2 (HER-2) and Epidermal Growth Factor Receptor (EGFR).[9-11] For multi-cellular organisms, this process

DOI: 10.5530/jyp.2024.16.92

is both crucial and necessary because cell nuclei depend on it for the best possible cellular activity. Polypeptides comprise the growth factors, which aid in the processes of proliferation and/or differentiation in both tumor and normal cells.[12] One of the most effective targets for many cancer treatments, including those for breast cancer, is EGFR. Cyclin-dependent kinases, or CDKs, play a critical role in numerous vital activities, including transcription, the cell cycle, communication, metabolism, and apoptosis. Any step of the cell cycle or transcription that is dysregulated causes apoptosis; but, if left unchecked, it can cause a host of illnesses, including cancer, stroke, and neurological diseases like Parkinson's or Alzheimer's disease. Drugs that inhibit CDK have been developed as cancer treatments.[13] Mammalian cells control their proliferation through a process known as the cell cycle, which includes the S, M, G2, and G1 phases. Quinazoline compounds are a preferred class of multi-agent therapeutics in the domains of biology and pharmacology. This scaffold was crucial among the several therapeutic agents due to its simple fabrication and wide range of pharmacological activity.[14] A large number of the chemotherapeutic drugs currently in use have low levels of selectivity and are quite toxic. They also cause the emergence of treatment resistance. Therefore, the development of highly selective, low-side-effect targeted chemotherapy drugs is necessary for the treatment of cancer. Quinazolines is an essential scaffold that has been connected to a number of biological processes (Figure 1). One of this scaffold's most notable biological functions is its anticancer properties. Numerous well-known quinazolines with anticancer properties operate on diverse molecular targets *via* distinct methods.[15] The present study is planned to the present study is planned to design the new series of substituted Quinazolinone derivatives and synthesize, carry out molecular docking studies for the proposed compounds against EGFR TK-PDB: 1M17 and CDK-PDB: 2KW6 receptor by using PyRx virtual screening tools Auto-dock vina software 0.9 version and to screen the compounds for their *in vitro* Cytotoxicity against MCF-7 breast cancer cell line.

MATERIALS AND METHODS

Experimental procedure for synthesis of Quinazolin-4-one

Ortho hydroxy benzoic acids in aromatic amine were refluxed in 100 mL of RBF and heated for 30 min to yield the intermediate product 2-hydroxy phenyl salicylamide. In ethanol, the intermediate product was mixed with aromatic aldehyde. 2-3 hr was spent refluxing the reaction mixture and leaving it overnight to create 2, 3-diphenyl 23-dihydro benzoxaine4-one. After dissolving the resultant 2, 3-diphenyl 2, 3-dihydro benzoxaine-4-one in ethanol, sulphonamide is added and refluxed for one hour. After adding ice cubes to create a solid product, filter the mixture to extract derivatives of dihydro quinazolin-4-one. The product was recrystalized from ethanol while the reaction condition was kept under TLC control. Using open capillaries, the melting point of the produced compounds was determined. Using silica G plates, TLC was used to regularly check the homogeneity and purification of the synthesized derivative. The iodine chamber was used for visualization and the mobile phase consisted of benzene and chloroform. The synthesized Quinazolinone' infrared range was expected at 4000-400 cm^{-1} using KBr discs scheduled for JASCO 4100 FTIR. An ethereal revision to the NMR spectrometer was prepared using DMSO-regents by JOEL FX90Q during the Fourier transform. Figure 2 showing the scheme of experimental reaction for selected methodology of research.

Molecular docking

The Auto Dock vina tool was obtained from the Pyrx virtual screening Tools, and Docking tests were conducted using these tools. The macro molecule of PDB set-up translation was done using Swiss Dock, and the ligand to PDB configuration modification was completed using Chem 3D pro 8.0. The RCSB website provided access to the Protein Data Bank Archive on the crystal structures of human CDK II (PDB ID: 2KW6) and EGFR-TKs (PDB ID: 1M17). Optimization of ligands and macro molecules is combined with regulation. Determine the root mean square deviation scores by resolving the essential amino acids into binding positions and allowing the docking procedure for docked ligands attentive to the organized binding position of pertinent enzymes. Every distinct compound at the desired dynamic position will be given a chance to dock. Each docking candidate was ranked according to how well it performed in the Ligand Binding Pocket (LBP) and the necessary interaction strategy.

Anticancer activity

The inhibitory concentration was determined using the MTT method (IC_{50}). The cancer cells were cultivated in a 96-well plate at a density of 1×104 cells per well for 48 hr in order to reach 80% confluency. Fresh media containing sample QS1 at different doses was added to the old medium, and it was left for a full day. Following the removal of the culture media from each well, 100 µL MTT was applied, and the wells were then incubated for 4 hr at 37°C. Following the extraction of the supernatant from every well, 100 µL of DMSO was introduced and allowed to stand for 10 min in order to dissolve the formazan crystals. The optical density was measured at 520 nm (ROBONIK, India) using ELISA multi-well plate reader.[16,17] IC_{50}-Values of respective Compounds (at 24 hr).

The % viability was calculated using the following formula in light of the findings:

% of viability=(OD value of experimental control-OD value of experimental sample/OD value of experimental control)×100.

Theexperiment was carried out triplicate. The analysis was conducted using SPSS version 17.0, a statistical software. A *p* value of less than 0.01 was deemed significant.

RESULTS

Molecular docking analysis

In order to mark the latent applicant under pro monitoring of malignancy for that purpose, newly synthesized molecules of quinazolin-4-one derivatives were processed using molecular docking under the binding pocket enzymes EGFR TK and CDK-II (PDB ID:1M17,2KW6) presented in Figure 3a and 3b. Furthermore, a range of journalistic sources claim that quinazolin-4(3H)-one related moieties have initiated to inhibit EGFR-TK. Among the primary reflective targets of Tyrosine Kinases (TK) inhibitors, the epidermal growth factor receptor plays a critical role in compartment extension in this instance. CDK-II may include a major duty in the G2 phase of the unit succession. What role does Cyclin Dependent Kinase-II (CDK-II) play in the evolution of the cell phase in resistance to cancer and other stressful-proliferative process. To investigate the binding affinity of quinazolin-4-ones against PDP ID: 1M17, where the EGFR is located. All of the synthesized compounds (QS1-QS4) were docked against the target protein cancer, and their ranking was determined based on their docked values. The reference molecules against CDK's Enzyme were doxorubicin with docked score values of -4.9 and -7.4 kcal/mol. Compounds that show a docking score of 7.0 or higher are often regarded as the superior agent for suppressing tumor activity. A thorough evaluation may be carried out and summarized in Table 2, which represents the inventory of active chemicals later obtained via docking investigations. The compounds that are produced, active, and docked have outstanding docked values exceeding 7.0 kcal/mol. The selection of 4 (QS1-4) compounds was based on factors such as druglikeness, QS1 against the (PDB:1M17) docked score, binding affinity between 1M17 and 2KW6, as well as findings from molecules of quinazolin-4-one derivatives that have been synthesized further against EGFR TK (1M17) QS1 has the greatest docked value (-10.1kcal/ mol) and the outstanding docked value (-8.7 kcal/ mol) against Cyclic Dependent Kinase II of cancer activity (2KW6). The selected Quinazolin-4-one moieties (QS1-4) on receptor sites and amino acid residues of Cyclin-dependent kinases (2KW6) and Epidermal growth factor tyrosine kinase (1M17) interactions presented in Table 4. Figure 3c represents the best binding pose as well as 2D structure of selected quinazolinones (QS1-4) interaction in EGFR-TK's receptor (1M17) and CDK(2KW6).

Molecular Interaction Analysis

Druglikeness, ADMET and Bioactivity prediction

ADME characteristics play a crucial role in the success or failure of candidate compounds in medication development. Diminished characteristics may cause the molecules to become more visible to the intended enzyme. One further crucial factor that frequently overshadows ADME activities is toxicity. When evaluating the bioavailability of medications that are vocally treated, Lipinski's rule is useful. The newly created compounds' chemical characteristics and bioactivity were examined using Mol inspiration to determine how similar they were to drugs Moreover, admet SAR datasets were used to estimate ADMET properties. According to Veber's criteria, every newly developed compound has a TPSA of no more than 140 and rotatable bonds of fewer than 10, which suggests that the compounds may have good oral absorption. Twenty. All of the new quinazolin-4-one drug's similarity qualities are represented in Tables 1 and 2. Good intestinal absorption is indicated by a Human Intestinal Absorption value of 0.9 or above. AMES toxicity evaluation is used to determine if a drug is carcinogenic or not. All synthesized compounds (1-4) that have been influenced have negative values, meaning they are not carcinogenic or mutagenic. Additionally, designed compounds have shown lower LD_{50} values (which are measures to causes of 50% trial residents). Additionally, it was discovered that the LD_{50} range (Given in Table 2) was somewhat higher and might be considered safe. The bioactivity value of

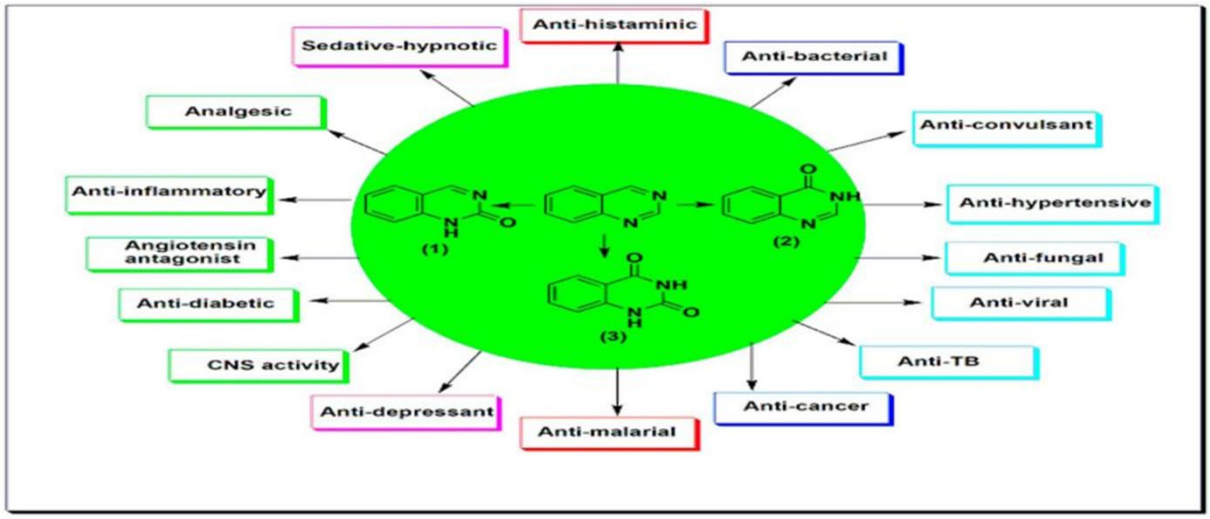


Figure 1: Quinazolin-4-one various biological action.

Table 1: ADMET properties of Quinazolin-4-one derivatives.

Comp. Code	MW	Log P	HBA	HBD	TPSA	Nrb	No. of violations
QS1	518.526	3.10	7	1	137.91	5	1
QS2	507.974	4.52	5	1	92.09	4	1
QS3	530.562	2.03	7	1	147.14	6	2
QS4	520.01	3.41	5	1	101.32	5	1

Table 2: Binding affinity and Physico-chemical action of potent moieties in the rules of druglikeness.

Comp. Code	Binding Affinity (kcal/mol)		HIA	BBB	Ames Toxicity (Yes/No)	Carcinogenicity	LD_{50} in rat (mol/kg)
	1M17	2KW6					
QS1	-10.1	-8.7	100	-1.529	Non-toxic	Non-Carcinogenic	2.617
QS2	-10.2	-8.8	98.928	-1.196	Non-toxic	Non-Carcinogenic	2.554
QS3	-9.8	-8.1	98.72	-1.449	Non-toxic	Non-Carcinogenic	2.958
QS4	-9.7	-8.8	100	-1.118	Non-toxic	Non-Carcinogenic	3.276

Table 3: Bioactivity score of proposed molecules of Quinazolin-4-derivatives.

C code	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease Inhibitor	Enzyme Inhibitor
QS1	0.26	0.45	0.27	0.36	0.15	0.11
QS2	0.17	0.39	0.19	0.33	0.09	0.07
QS3	0.29	0.52	0.31	0.38	0.17	0.15
QS4	0.20	0.44	0.23	0.35	0.11	0.09

the synthesized Quinazolinone were also well represented in Table 3 such as G-protein coupled receptor ligands, ion channel modulators, nuclear receptor ligands, kinase inhibitors, protease inhibitors, and enzyme inhibitors.

Anticancer study

Based on a motivated related study of Quinazolinone prospects' (QS1) anticancer properties against MCF-7 human carcinoma cells utilizing positive control, our current investigation was conducted. The data generated will be used to construct a dose response curve that shows the concentration of the evaluation molecule required to kill 50% of the cell population (IC_{50}). Figure 4a presents the IC_{50} of Quinazolinone at different concentrations (QS1) against breast cancer cell lines, along with the feasibility and ethics of the study with graphical representation. Figure 4a and 4b findings demonstrated that Quinazolinone (QS1) have a heightened Cytotoxicity against MCF-7 cancer cell lines, with corresponding IC_{50} values compared to positive control dead cells

Chemistry

QS1: yield: 88.71%, M.P:210, R_f 0.84: IR (KBr, vmax, cm^{-1}):1628.77(C=O), 3291.00(C-H), 3552.45(N-H), 1504.14(N=O), 1467.86(C-F), 1182.70(S=O).

QS2: yield: 75.39%, M.P:201, R_f 0.74: IR (KBr, vmax, cm^{-1}):1670.47(C=O), 3210.51(C-H), 3445.64(N-H), 1143.24(Cl), 1380.34(C-F).

QS3: yield: 80.11%, M.P:224, R_f 0.84: IR (KBr, vmax, cm^{-1}):1670.47(C=O), 3210.51(C-H), 3445.64(N-H), 1143.24(N=O), 1380.34(O-CH).

QS4: yield: 870.79%, M.P:215, R_f 0.70: IR (KBr, vmax, cm^{-1}):1651.47(C=O), 3230.15(C-H), 3629.29(N-H), 1441.11(Cl), 2852.32(O-CH_3), 1207.21(S=O).

DISCUSSION

Over expression of EGFR has been linked to various cancers, including those of the brain, bladder, head, lung, colon, breast, ovarian, and prostate. Additional key points have been made since quinazolines, purines, and pyrimidines are effective CDK enzyme inhibitors.[18,19]

The molecular docking of the designed molecules against the target protein EGFR (1M17), Cyclic dependent Kinase (2KW6), using docking software Auto dock vina (PyRx), also performed computational studies like Lipinski rule of 5 for druglikeness, admet SAR and pkCSM for Pharmaco-dynamic, kinetic properties and synthesis of selected lead molecules by conventional method based on their least binding affinities and

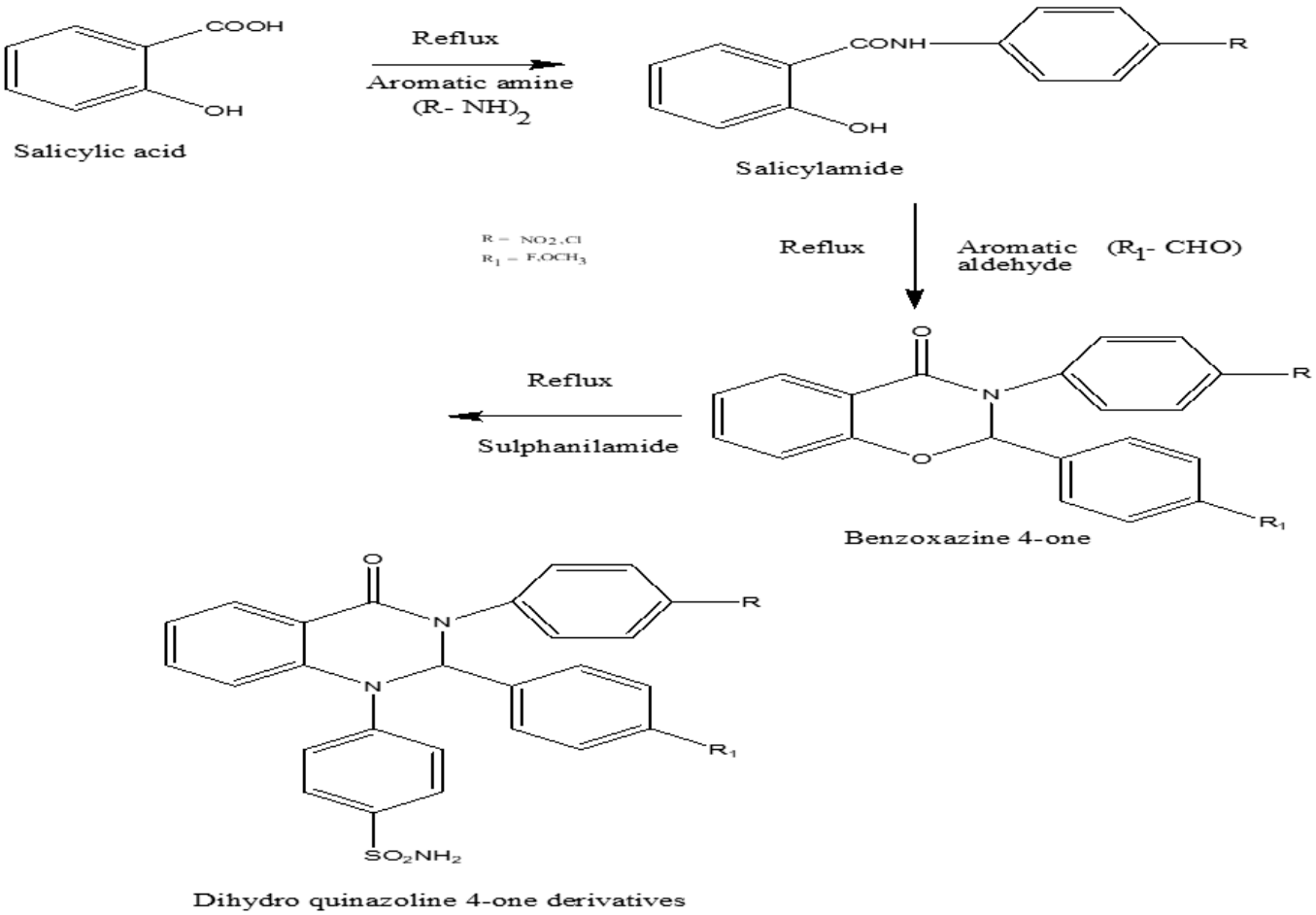


Figure 2: General Scheme.

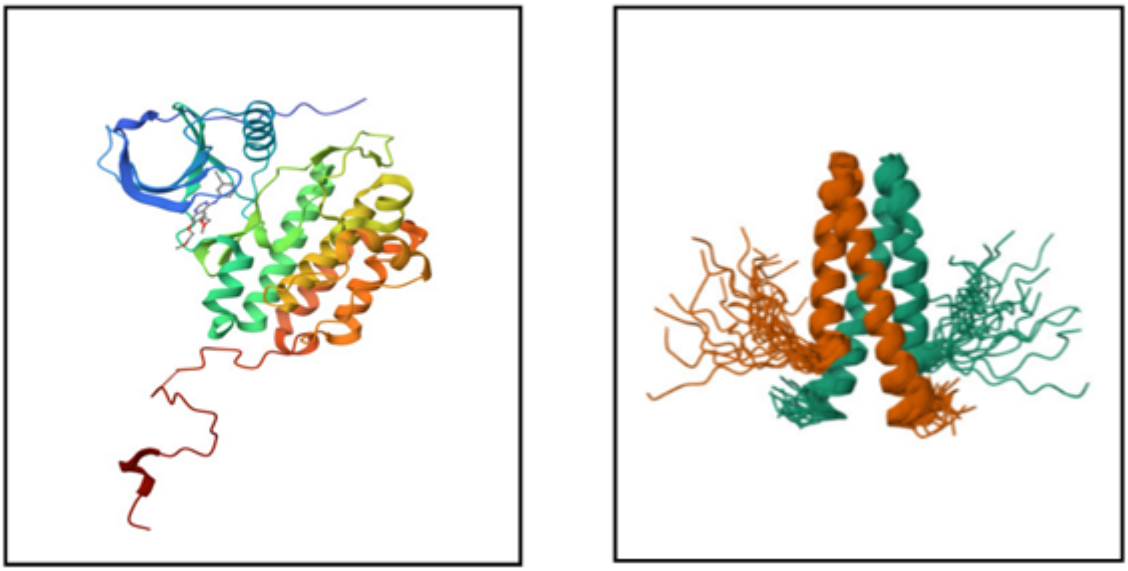
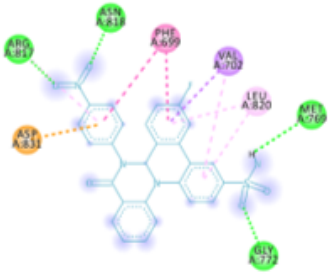
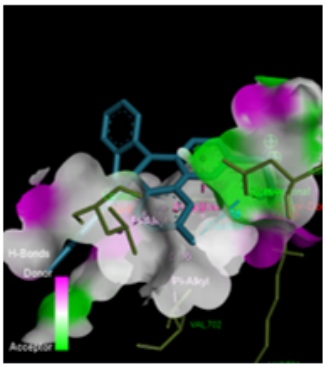
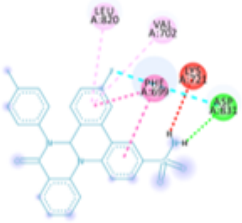
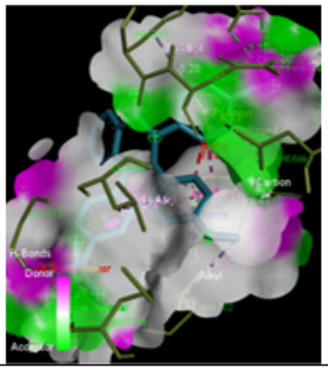
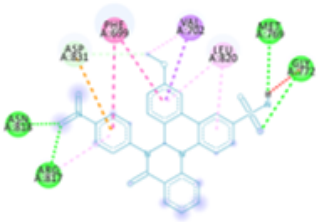
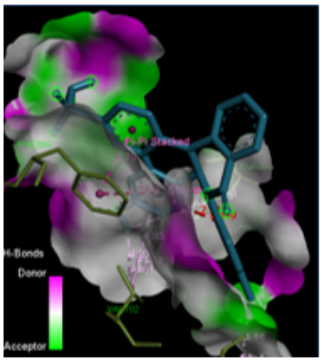
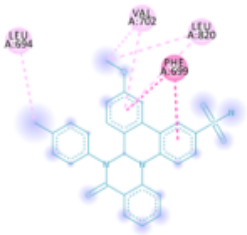


Figure 3: (a) EGFR TK (b) CDK.

Comp. Code	3D Binding Pose	2D Interaction
QS1-1M17		
QS2-1M17		
QS3-1M17		
QS4-1M17		

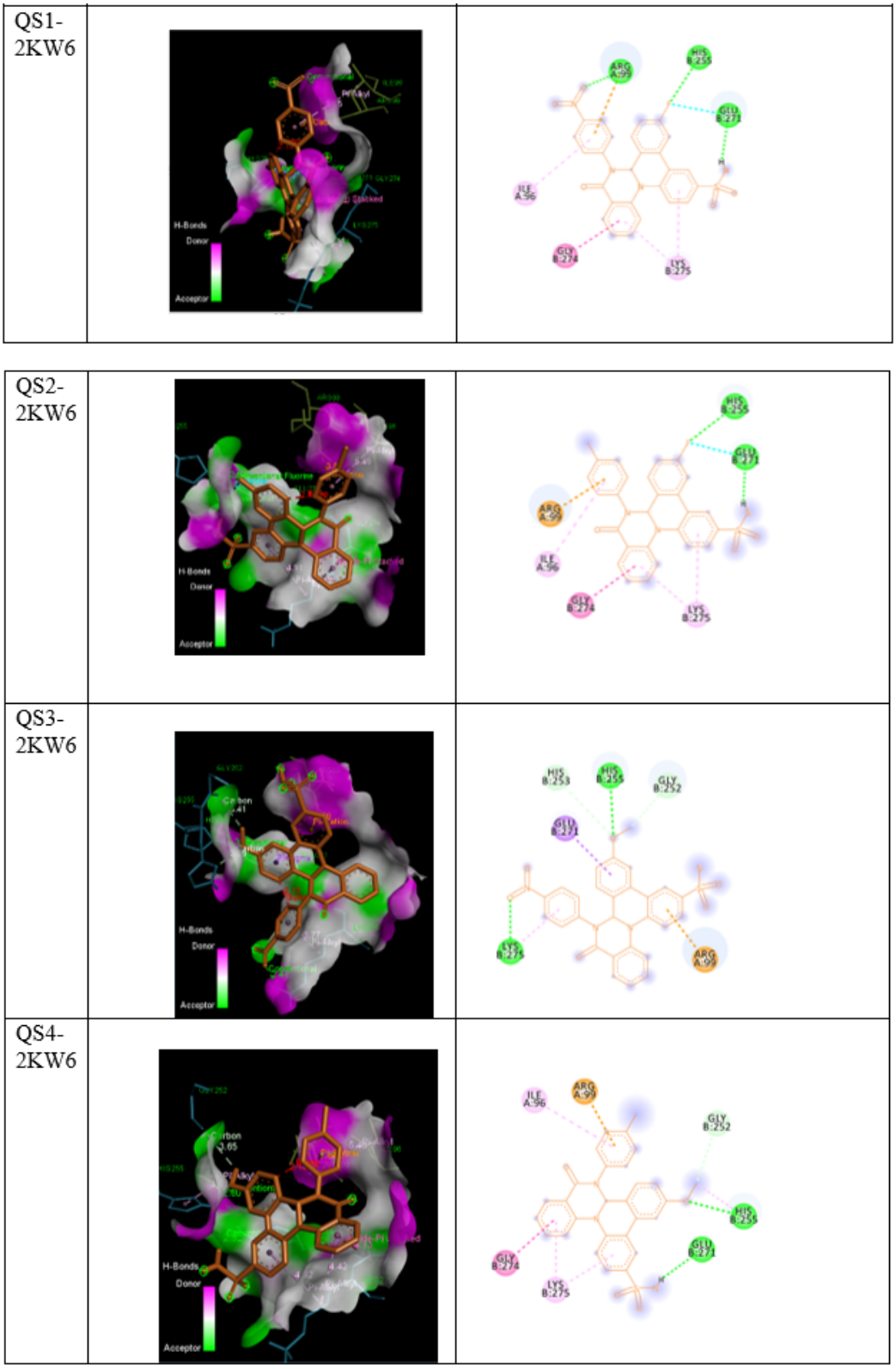


Figure 3c: Best Binding pose and 2D structure of Quinazolinones interaction in EGFR-TK's receptor (1M17) and CDK(2KW6).

Table 4: Interactions between produced Quinazolin-4-one moieties on receptor sites and amino acid residues of Cyclin-dependent kinases (2KW6) and Epidermal growth factor tyrosine kinase (1M17).

Code	Hydrogen bond		Pi -alkyl		Pi-sigma	
	1M17	2KW6	1M17	2KW6	1M17	2KW6
QS1	ARG A:817 ASN A:818 MET A:769 GLY A:772	ARG A:99 HIS B:255 GLU B:271	LEU A:820	ILE A: 96 LYS B: 275	VAL A:702	----
QS2	ASP A:831	HIS B: 255 GLU B: 271	LEU A:820 VAL A:702	ILE A: 96 LYS B: 275	----	-----
QS3	ARG A:817 ASN A:818 MET A:769 GLY A:772	LYS B: 275 HIS B: 255	LEU A:820	----	VAL A:702	GLU B:271 ARG A:99(PI cation)
QS4	------	HIS B: 255 GLU B271	LEU A:820 VAL A:702	ILE A: 96 LYS B: 275	------	ARG A:99(PI cation)

Drug concentration(ug/ml)	% Cytotoxicity	
	DOX	QS1
0	95.8	97.2
20	52.4	71.7
40	43.9	52.4
60	30.6	47.8
80	25.4	47.8
100	22.2	40.3
MCF-7Cells (IC50)	21±0.7	40±0.5

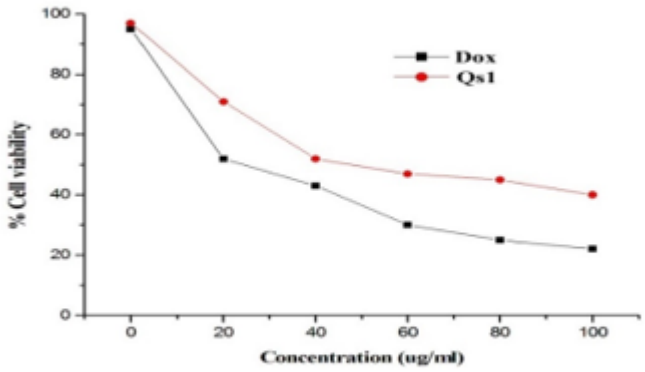


Figure 4a: Cytotoxicity against a breast cancer cell line using the MTT Assay technique.

their characterization by spectral analysis.[20] The selected lead molecules (QS1) were synthesized by conventional method with satisfactory yield.

The aforementioned compounds were created through 3-4 distinct reaction steps, yielding a product that was 75% to 86%. The melting point was determined by the open capillary tube method and the structures of the synthesized Quinazolinone were determined using an Infra-red spectral analysis. The completion of the reaction conditions was tracked using TLC chromatography.[21,22]

Based on the aforementioned investigation, docking poses and their corresponding binding pockets were generated based on docking parameters. Current research is ostensibly conducted in cooperation with the fastening relationships above the protein under attack. Preferred quinazolin-4-one derivatives can be extracted using molecular docking analysis, which also removes the binding affinities of some candidates, resulting in binding values of -7.1 and -9.0 kcal/mol, respectively, as well as reference drugs 1 and 2.[23] Because every single one of the suggested compounds has a feasible site into the intended macromolecule, they are all positioned to successfully latch onto the cancer inhibitors EGFR and CDK's Enzyme.[24] The outcome of the docking exploration is shown to contain lower energy values (higher binding energy values) for each distinct docking moieties. Compounds that show a docking score of 7.0 or higher are often regarded as the superior agent for suppressing tumor activity.[25] Along with the various interface scores of the chosen molecules (QS1-QS4). Showcase the

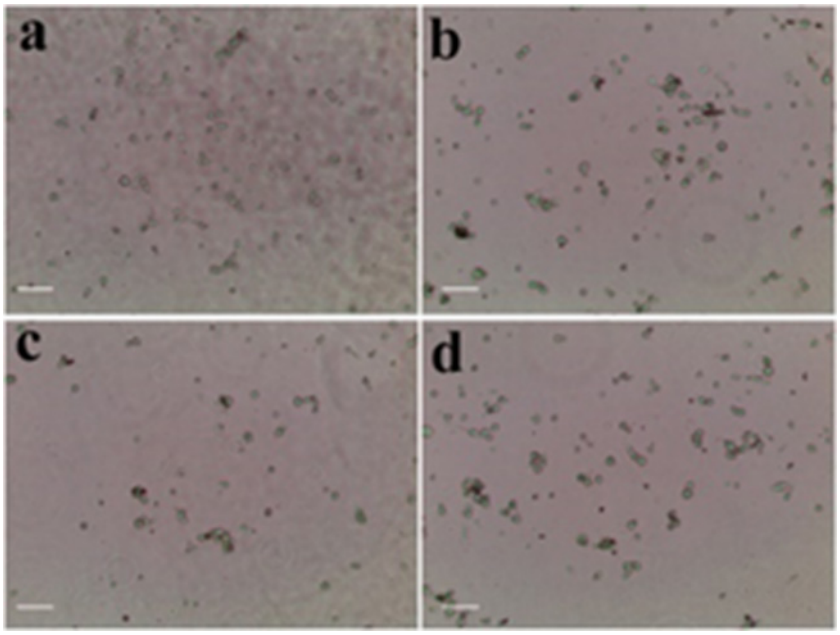


Figure 4b: Mtt assay images showing (a-d) the intensity of cells at different concentration.

docking molecule candidates' largely exceptional lower binding energy (higher binding energy) results.[26] The substituted F-NO2 and F-Cl ligand (QS1 and QS2 generated the majority efficient at the high binding score's centre of -10.1 kcal/mol, -10.2 kcal/mol (EGFR) and -8.7 kcal/mol, -8.8 kcal/mol (CDK), along with (1-4) Ligands in order to be docked with the enzyme EGFR TK's and CDK'S. All of the substituted ligand's O-CH3 against the EGFR and CDK'S enzymes exhibited good docking scores greater than -7.0 kcal/mol. Refusal infraction among all the ligands that were chosen shows signs of drug similarity.[27] In addition, the elected Quinazolinones remaining over the candidates had excellent Blood Brain Barrier (BBB), Intestinal Absorption (HIA), rejection carcinogenicity, AMES negative, and bioavailability. the docking ligand's hydrogen bonding, electrostatic interaction, and Pi sigma and Pi alkyl interactions. Observing how ligand interactions bond reflectively in the quinazoli-4-one's hub of activity to block the activity of anticancer enzymes.

The selected lead compounds were synthesized and evaluated for *in vitro* cytotoxic activity against human cancer cell lines MCF-7 using doxorubicin as standard drug by MTT Assay method and IC_{50} values were calculated. Average cell viability was calculated, and graph plotted concentration in X axis versus % viability in Y axis. Lead compounds QSD1 exhibited potent cytotoxic activity against breast (MCF-7) cancer cell line with IC_{50} value 40±0.5 µg/mL respectively as compared to the standard drug doxorubicin. The compounds QS1 exhibited significant cytotoxic activity against breast (MCF-7) cancer cell line with compared to the reference drug. The tested compounds possess good anti-cancer activity against all the concentrations. The percentage of cytotoxicity is directly proportional to the concentration.[28,29]

The various electron donating and withdrawing group of 4-substituted aromatic aldehyde substituted at C-3 position of Quinazolinone. Substitution of electron donating group 4-OCH_3 and electron withdrawing group F of Quinazolinone at C-3 position demonstrated better *in vitro* cytotoxic activity as compared to standard drug.

Substitution of electron withdrawing group NO_2 and Cl, F sulfanilamide of Quinazolinone at C-3, C-2 position with demonstrated better *in vitro* cytotoxic activity against tumor cell lines as compared to standard.[30] The present study states that QS1 lead compounds of Quinazolinone were substituted the electron-withdrawing group NO_2 at C-3 position and fluoro at C-2 of phenyl Quinazolinone ring exhibited significant cytotoxicity action against human breast cancer (MCF-7) cell line as compared with used doxorubicin as standard drug.

CONCLUSION

The current work includes a subset of quinazolin-4-one derivative compounds and then uses PDB IDs 1M17 and 2KW6 to conduct a docking analysis to investigate the binding relationship between EGFR and CDK. Numerous synthesized quinazolinone compounds that were suggested were proven to contain certain moieties that produce an excellent range of binding scores. The most effective substance has a high docking score of -9.7 kcal/mol to -10.2 kcal/mol (EGFR) and -8.1 kcal/mol to -8.8 kcal/mol (CDK), According to data from molecular docking studies. The compounds were selected according to their docking scores and physiochemical characteristics. Additional investigation reveals that the selected compounds strongly inhibit both CDK and EGFR, which could lead to the development of more effective quinazolin-4-one derivatives. The MCF-7 breast cancer cell line,

which produces concentration rises and cell viability declines, was also evaluated for cytotoxicity *in vitro* in this study. This study demonstrates the necessity of synthesizing and testing a further series of compounds for their potential to cause breast cancer.

ACKNOWLEDGEMENT

The authors are grateful and immensely thankful to Management and principal, Prime college of pharmacy, Palakkad, Kerala, for the given necessary facility. The authors are gratified headed for the principal also management of Karpagam College of Pharmacy, Coimbatore, in favour of given that crucial infrastructure**.**

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EGFR: Epidermal Growth Factor Receptor; **TK's:** Tyrosine Kinases; **CDK:** Cyclic-dependent kinases; **IR:** Infra-red; **TLC:** Thin layer Chromatography; **DMSO:** Di-methyl Sulphoxide; **PDB:** Protein data bank; **PBS:** Phosphate buffered cell line suspension; **IC_{50}:** Half maximal inhibitory concentration; **GPCR:** G-Protein coupled receptor; **B.B.B:** Blood brain barrier; **TPSA:** Topical polar surface area; **2D:** Two-dimensional; **3D:** Three dimensional; **MTT:** 3(4,5 Di-methyl thiazol-2yl) 2,5 diphenyl Tetrazolium Bromide; **OD:** Optical density; **HIA:** Human intestinal absorption; **MW:** Molecular weight; **nRB:** Number of rotatable bond; **HBD:** Hydrogen bond donor; **HBA:** Hydrogen bond acceptor.

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Cite this article: Kavitha K, Jothikanth V, Kaniga P, Kumar MS, Revathi P, Esakiprasanth R, *et al*. Synthesis, Molecular Docking, *in silico* druglikeness: *In vitro* Cytotoxicity Study on MCF-7 Cell Line of Quinazolin-4-one Scaffold. J Young Pharm. 2024;16(4):725-34.