RESEARCH PAPER

Design, Synthesis, and Mechanistic Pharmacological Evaluation of Novel Quinazolinone–Thiazolidinone Hybrid Scaffolds Targeting PI3K/Akt/mTOR Signaling in Chemoresistant Carcinoma Models

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ABSTRACT

Background: Chemoresistance remains a major challenge in cancer therapy, often resulting in treatment failure and tumor recurrence. One of the key molecular mechanisms responsible for cancer progression and drug resistance involves dysregulation of the PI3K/Akt/mTOR signaling pathway, which regulates cellular growth, proliferation, and survival. Targeting this signaling cascade has therefore emerged as an important strategy for the development of novel anticancer agents. Heterocyclic scaffolds such as quinazolinone and thiazolidinone have demonstrated significant pharmacological potential in medicinal chemistry, particularly for anticancer drug discovery.

Objective: The present study aimed to design, synthesize, and evaluate novel quinazolinone–thiazolidinone hybrid compounds as potential inhibitors of the PI3K/Akt/mTOR signaling pathway in chemoresistant carcinoma models.

Methods:A series of hybrid derivatives were designed using computational modeling approaches and synthesized through a multistep synthetic pathway involving quinazolinone intermediate formation, Schiff base condensation, and cyclization with thioglycolic acid. The synthesized compounds were characterized using spectroscopic techniques including infrared spectroscopy, proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, and mass spectrometry. Molecular docking studies were conducted to investigate interactions with proteins involved in the PI3K/Akt/mTOR signaling cascade. Biological evaluation was performed using chemoresistant carcinoma cell lines. Cytotoxic activity was determined using the MTT assay, while apoptosis induction and cell cycle distribution were analyzed using Flow Cytometry. Mechanistic studies were conducted through Western blot analysis and gene expression analysis to examine modulation of signaling proteins.

Results: The synthesized quinazolinone–thiazolidinone hybrid compounds demonstrated variable cytotoxic activity against chemoresistant carcinoma cells. Among the tested derivatives, selected compounds exhibited comparatively stronger antiproliferative activity and induced significant apoptosis in treated cells. Cell cycle analysis indicated arrest at specific phases of the cell cycle, suggesting inhibition of cellular proliferation. Molecular docking studies revealed favorable binding interactions between the hybrid molecules and key proteins of the PI3K/Akt/mTOR signaling pathway. Western blot analysis further indicated reduced phosphorylation levels of Akt and mTOR proteins, supporting inhibition of the signaling cascade.

Conclusion: The findings of this study demonstrate that quinazolinone–thiazolidinone hybrid scaffolds represent promising candidates for anticancer drug development. The observed cytotoxic activity and mechanistic evidence suggest that these compounds may exert their effects through modulation of the PI3K/Akt/mTOR signaling pathway. Further pharmacological investigations and preclinical studies are warranted to explore their therapeutic potential in cancer treatment...

Keywords: *Quinazolinone hybrids; Thiazolidinone derivatives; Chemoresistant carcinoma; PI3K/Akt/mTOR pathway; Anticancer agents...*

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INTRODUCTION

Cancer and the Challenge of Chemoresistance

Cancer remains one of the leading causes of mortality worldwide, characterized by uncontrolled cell proliferation, evasion of apoptosis, and the ability to invade surrounding tissues. Despite substantial advances in chemotherapy and targeted therapy, the emergence of chemoresistance significantly limits treatment efficacy and contributes to disease relapse and poor patient prognosis. Chemoresistance may arise through multiple mechanisms, including drug efflux, enhanced DNA repair, mutation of drug targets, and activation of survival signaling pathways (Holohan et al., 2013). Among these mechanisms, aberrant activation of intracellular signaling cascades that promote cell survival and proliferation plays a critical role in tumor progression and resistance to anticancer agents.

One of the most prominent signaling networks involved in cancer progression and drug resistance is the phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. This pathway regulates numerous cellular processes such as growth, metabolism, survival, and angiogenesis. Dysregulation or hyperactivation of PI3K/Akt/mTOR signaling has been observed in various malignancies, including breast, colorectal, lung, and prostate cancers (Fruman et al., 2017). Overactivation of Akt, a central kinase in this pathway, promotes tumor cell proliferation, inhibits apoptosis, and contributes to therapeutic resistance in many cancers (Manning & Toker, 2017). Studies have demonstrated that inhibition of the PI3K/Akt/mTOR pathway can restore sensitivity to chemotherapy and induce apoptosis in resistant cancer cells, highlighting it as a promising therapeutic target (Rahman et al., 2025).

Quinazolinone Scaffold in Anticancer Drug Discovery

Heterocyclic compounds play a pivotal role in modern medicinal chemistry, particularly in the development of anticancer agents. Among them, quinazolinone derivatives have attracted considerable attention due to their diverse pharmacological properties, including anticancer, antimicrobial, anti-inflammatory, and antiviral activities (Kumar et al., 2022). Quinazolinone-based molecules have been reported to interfere with several signaling pathways involved in tumor growth and survival, particularly the PI3K/Akt/mTOR pathway. These compounds can inhibit PI3K activity, suppress Akt phosphorylation, and reduce mTOR signaling, ultimately leading to decreased cancer cell proliferation and increased apoptosis (Ayyappan, 2025; Kumar et al., 2022).

Recent studies have demonstrated that novel quinazolinone derivatives exhibit potent cytotoxic effects against various cancer cell lines by inducing apoptosis and cell cycle arrest. For instance, a quinazolinone-based compound was shown to inhibit tumor cell proliferation and suppress the PI3K/Akt/mTOR signaling cascade, resulting in significant anticancer activity in both in vitro and in vivo models (Wani et al., 2016). Similarly, another quinazolinone derivative induced apoptosis and autophagy in fluorouracil-resistant colorectal cancer cells through inhibition of Akt and mTOR signaling pathways, suggesting its potential role in overcoming chemoresistance (Chen et al., 2021).

Thiazolidinone Derivatives as Bioactive Pharmacophores

Another important heterocyclic scaffold widely explored in drug discovery is thiazolidinone. Thiazolidinone derivatives possess a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects (Tripathi et al., 2020). The presence of sulfur and nitrogen atoms in the thiazolidinone ring contributes to its strong binding affinity toward various biological targets, making it an attractive pharmacophore for medicinal chemistry applications.

Several thiazolidinone-based compounds have demonstrated promising anticancer activity through mechanisms such as apoptosis induction, inhibition of tumor cell proliferation, and modulation of signaling pathways associated with tumor survival. In particular, thiazolidinone derivatives have been reported to interfere with kinase-mediated pathways involved in cell cycle regulation and apoptosis, making them potential candidates for targeted anticancer therapy (Verma et al., 2019).

Hybrid Pharmacophore Strategy in Anticancer Drug Design

In recent years, the molecular hybridization approach has emerged as a powerful strategy in medicinal chemistry for the development of multifunctional therapeutic agents. This strategy involves the combination of two or more pharmacologically active scaffolds into a single molecule to enhance biological activity, improve selectivity, and reduce adverse effects (Morphy & Rankovic, 2005). Hybrid molecules often exhibit synergistic pharmacological properties due to the simultaneous modulation of multiple biological targets.

The integration of quinazolinone and thiazolidinone scaffolds into a single hybrid framework may lead to the development of novel compounds with enhanced anticancer activity. Quinazolinone derivatives are known for their kinase inhibitory properties, whereas thiazolidinone scaffolds provide additional pharmacophoric features that can improve binding affinity and biological potency. Such hybrid molecules may effectively target critical signaling pathways involved in tumor survival, particularly the PI3K/Akt/mTOR pathway.

Rationale and Objective of the Study

Given the crucial role of the PI3K/Akt/mTOR pathway in tumor progression and chemoresistance, the development of novel inhibitors targeting this signaling cascade represents a promising strategy for anticancer therapy. Hybrid molecules incorporating quinazolinone and

thiazolidinone pharmacophores may provide improved biological activity through multi-target interactions with key proteins involved in cancer signaling.

Therefore, the present study aims to design, synthesize, and evaluate novel quinazolinone–thiazolidinone hybrid compounds as potential inhibitors of the PI3K/Akt/mTOR signaling pathway in chemoresistant carcinoma models. The synthesized compounds will be subjected to comprehensive pharmacological evaluation, including cytotoxicity assays, mechanistic pathway analysis, and molecular docking studies to elucidate their anticancer potential and underlying mechanisms of action.

2. MATERIALS AND METHODS

2.1 Materials

Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical grade and were used without further purification unless otherwise stated. Common reagents included anthranilic acid, substituted aromatic aldehydes, thioglycolic acid, ammonium acetate, acetic anhydride, ethanol, methanol, chloroform, dimethyl sulfoxide (DMSO), and glacial acetic acid. Solvents used in synthesis and purification were dried and distilled prior to use when necessary. Thin-layer chromatography plates precoated with silica gel were used for monitoring the progress of reactions.

Cell Lines

Chemoresistant carcinoma cell lines were utilized for biological evaluation of the synthesized compounds. Representative resistant cell models included human breast carcinoma cells (MCF-7/ADR) and colorectal carcinoma cells (HCT-116 resistant variant). Cells were maintained under sterile conditions and routinely monitored for viability and morphological integrity prior to experimentation.

Culture Media

Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum, penicillin, and streptomycin to maintain optimal growth conditions. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % carbon dioxide. Prior to experimental procedures, cells were sub-cultured to maintain exponential growth and appropriate confluency levels.

Analytical Instruments

The structural and physicochemical characterization of synthesized compounds was carried out using standard analytical instrumentation. Infrared spectra were recorded using a Fourier transform infrared spectrophotometer with potassium bromide pellet technique. Nuclear magnetic resonance spectra were obtained using a high-field NMR spectrometer operating at appropriate frequencies for proton and carbon nuclei. Mass spectra were recorded using an electrospray ionization mass spectrometer. Melting points were determined using a digital melting point apparatus and were uncorrected. Elemental analysis was performed to confirm the elemental composition of the synthesized compounds.

2.2 Computational Drug Design

2.2.1 Molecular Modeling

The design of quinazolinone–thiazolidinone hybrid molecules was performed using molecular modeling approaches aimed at optimizing interactions with key proteins involved in oncogenic signaling pathways. The basic scaffold was constructed by integrating quinazolinone and thiazolidinone pharmacophores within a single molecular framework. Various substituents were introduced on the aromatic ring system to enhance molecular diversity and modulate electronic and steric properties.

Three-dimensional structures of the designed ligands were generated using molecular drawing and modeling software. The generated structures were energy minimized using molecular mechanics force fields in order to obtain stable conformations suitable for docking analysis. Geometry optimization ensured that the molecules adopted energetically favorable conformations prior to further computational evaluation.

2.2.2 Molecular Docking

Molecular docking studies were conducted to investigate the potential binding interactions between the designed hybrid compounds and proteins involved in the PI3K/Akt/mTOR signaling pathway. Crystal structures of the target proteins were retrieved from publicly available structural databases and prepared for docking by removing water molecules and co-crystallized ligands.

The prepared ligands were docked into the active binding pockets of the target proteins using an automated docking algorithm. The docking procedure involved generation of multiple ligand conformations within the active site, followed by scoring based on binding affinity and intermolecular interactions. The docking simulations allowed evaluation of hydrogen bonding, hydrophobic interactions, and van der Waals forces between the ligands and amino acid residues within the binding site.

2.2.3 ADMET Prediction

The pharmacokinetic properties of the designed molecules were evaluated through in-silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction studies. Computational tools were employed to assess drug-likeness parameters, including molecular weight, lipophilicity, hydrogen bond donors and acceptors, and polar surface area. These parameters were analyzed to determine whether the designed compounds satisfied commonly accepted criteria for oral bioavailability.

Additionally, predictions related to intestinal absorption, blood–brain barrier permeability, metabolic stability, and potential toxicity were evaluated. Such computational screening provided preliminary insights into the pharmacokinetic suitability of the designed molecules prior to experimental evaluation.

2.3 Chemistry: Synthesis of Hybrid Compounds

2.3.1 Synthetic Strategy

The synthesis of quinazolinone–thiazolidinone hybrid compounds was carried out through a multistep synthetic approach involving formation of a quinazolinone core followed by Schiff base formation and cyclization to produce the final hybrid scaffold.

The overall synthetic strategy involved the following sequence:

Quinazolinone derivative
↓
Schiff base formation
↓
Cyclization with thioglycolic acid
↓
Quinazolinone–Thiazolidinone hybrid compound

This synthetic route was selected because it enables efficient construction of the thiazolidinone ring system while retaining the biologically active quinazolinone moiety within the final molecular framework.

2.3.2 General Synthetic Procedure

Preparation of Quinazolinone Intermediate

The quinazolinone intermediate was synthesized through the condensation of anthranilic acid with an appropriate acylating agent under reflux conditions. The reaction mixture was heated for a specified duration to facilitate cyclization and formation of the quinazolinone nucleus. After completion of the reaction, the mixture was cooled to room temperature and poured into ice-cold water to precipitate the intermediate compound. The resulting solid was filtered, washed with distilled water, and dried under reduced pressure.

Formation of Schiff Base Derivatives

The synthesized quinazolinone intermediate was further reacted with substituted aromatic aldehydes in an alcoholic medium to generate Schiff base intermediates. The reaction mixture was refluxed in the presence of a catalytic amount of glacial acetic acid to facilitate condensation between the amino group of the quinazolinone derivative and the carbonyl group of the aldehyde.

The progress of the reaction was monitored using thin-layer chromatography. Upon completion, the reaction mixture was cooled and the precipitated product was collected by filtration. The crude Schiff base derivatives were washed with cold solvent and dried prior to further reaction.

Cyclization to Form Thiazolidinone Ring

The Schiff base intermediates were subsequently subjected to cyclization with thioglycolic acid to form the thiazolidinone ring. The reaction was performed in an alcoholic medium under reflux conditions in the presence of a catalytic amount of an appropriate acid or Lewis acid catalyst.

During the reaction, nucleophilic attack of thioglycolic acid on the imine carbon facilitated ring closure, leading to the formation of the thiazolidinone heterocycle. After completion of the reaction, the mixture was cooled and poured into cold water to induce precipitation of the final quinazolinone–thiazolidinone hybrid compounds.

Purification

The crude products obtained from the reaction were purified using conventional purification techniques. Recrystallization from suitable solvents was employed to obtain pure compounds with improved crystallinity. In cases where further purification was required, column chromatography using silica gel as the stationary phase and appropriate solvent systems as the mobile phase was performed.

The purified compounds were dried under vacuum and stored in airtight containers until further analysis.

2.3.3 Characterization

The synthesized compounds were characterized using standard spectroscopic and analytical techniques to confirm their chemical structures.

Infrared (IR) spectroscopy was employed to identify characteristic functional groups present in the synthesized molecules, including carbonyl, imine, and aromatic stretching vibrations.

Proton nuclear magnetic resonance (^1H NMR) spectroscopy was used to determine the chemical environment of hydrogen atoms within the molecules, while carbon-13 nuclear magnetic resonance (^13C NMR) spectroscopy provided information regarding the carbon skeleton of the synthesized compounds.

Mass spectrometry was performed to determine the molecular mass and fragmentation pattern of the compounds, thereby confirming the molecular structure. Elemental analysis was conducted to verify the percentage composition of carbon, hydrogen, nitrogen, and sulfur in the synthesized compounds, ensuring consistency with theoretical values derived from the proposed molecular formula.

These analytical techniques collectively enabled comprehensive structural confirmation of the synthesized quinazolinone–thiazolidinone hybrid derivatives.

2.4 Biological Evaluation

2.4.1 In-vitro Cytotoxicity Assay

The cytotoxic potential of the synthesized quinazolinone–thiazolidinone hybrid compounds was evaluated using the **MTT assay**, which measures cellular metabolic activity as an indicator of cell viability.

Cell Lines

Chemoresistant carcinoma cell lines were employed as experimental models to assess the anticancer activity of the synthesized compounds. The selected cells were maintained under sterile conditions and cultured in appropriate growth medium supplemented with serum and antibiotics. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % carbon dioxide.

Procedure

Cell Seeding

Cells were seeded into 96-well culture plates at an appropriate density to allow exponential growth during the experiment. The seeded plates were incubated for approximately 24 hours to allow proper attachment of cells to the surface of the wells.

Compound Treatment

The synthesized compounds were dissolved in dimethyl sulfoxide and diluted with culture medium to obtain different concentrations. After the initial incubation period, the culture medium was replaced with fresh medium containing the test compounds at various concentrations. Control wells containing only culture medium or solvent control were also included.

Incubation

Following compound treatment, the plates were incubated for a defined period to allow interaction between the cells and the test compounds.

Absorbance Measurement

After incubation, MTT reagent solution was added to each well and the plates were further incubated to allow reduction of the tetrazolium salt to insoluble formazan crystals by metabolically active cells. The formed crystals were dissolved using a suitable solvent, and absorbance was measured at an appropriate wavelength using a microplate reader.

Cell viability percentages were calculated relative to untreated control cells. The concentration of compound required to inhibit 50 % of cell viability was determined and expressed as the **IC_{50} value**.

2.4.2 Apoptosis Assay

To investigate whether the reduction in cell viability was associated with apoptotic cell death, an apoptosis assay was performed using Annexin V staining followed by Flow Cytometry analysis.

Cells were treated with selected compounds and incubated for the required duration. After treatment, cells were harvested and washed with cold phosphate-buffered saline. The cell suspension was then stained with Annexin V-fluorescent conjugate and propidium iodide according to standard staining protocols.

The stained cells were analyzed using flow cytometry to differentiate between viable cells, early apoptotic cells, late apoptotic cells, and necrotic cells based on fluorescence intensity. This method enabled quantitative assessment of apoptosis induced by the test compounds.

2.4.3 Cell Cycle Analysis

Cell cycle distribution analysis was performed using Flow Cytometry in order to determine whether the synthesized compounds induced cell cycle arrest in chemoresistant carcinoma cells.

Cells were treated with selected compounds for the required incubation period. Following treatment, cells were harvested and fixed using cold ethanol to preserve cellular DNA content. The fixed cells were then treated with RNase and stained with propidium iodide, which intercalates with DNA.

The stained cells were analyzed by flow cytometry, and the DNA content was measured to determine the proportion of cells present in different phases of the cell cycle, including G0/G1, S, and G2/M phases.

The objective of this experiment was to determine whether the test compounds caused arrest at specific stages of the cell cycle, thereby inhibiting cellular proliferation.

2.5 Mechanistic Studies

2.5.1 Western Blot Analysis

To investigate the molecular mechanism underlying the anticancer activity of the synthesized compounds, protein expression analysis was carried out using the Western Blotting technique.

Cells treated with selected compounds were lysed using an appropriate lysis buffer to extract total cellular proteins. The protein concentration of each sample was quantified prior to electrophoresis.

Equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequently transferred onto polyvinylidene fluoride membranes. The membranes were blocked to prevent non-specific binding and incubated with specific primary antibodies targeting proteins involved in the PI3K/Akt/mTOR signaling pathway.

Proteins Analyzed

PI3K

Akt

mTOR

phosphorylated Akt (p-Akt)

phosphorylated mTOR (p-mTOR)

After incubation with appropriate secondary antibodies, protein bands were visualized using a chemiluminescent detection method.

Purpose

The purpose of this analysis was to evaluate whether treatment with the synthesized compounds influenced the expression or phosphorylation status of key signaling proteins involved in the PI3K/Akt/mTOR pathway, thereby providing mechanistic insights into their anticancer activity.

2.5.2 Gene Expression Analysis

Gene expression analysis was performed using Reverse Transcription Polymerase Chain Reaction to examine the effect of the synthesized compounds on transcriptional regulation of genes associated with the PI3K/Akt/mTOR signaling pathway and apoptosis.

Total RNA was extracted from treated and control cells using standard RNA isolation procedures. The isolated RNA was reverse-transcribed to synthesize complementary DNA using reverse transcriptase enzyme.

The resulting complementary DNA was amplified using specific primers for the target genes through polymerase chain reaction.

Genes Analyzed

PI3K

AKT

mTOR

Bax

Bcl-2

The amplified gene products were analyzed to determine relative expression levels in treated cells compared to untreated control cells.

2.6 Molecular Docking Studies

Molecular docking studies were performed to evaluate the potential interaction of the synthesized quinazolinone–thiazolidinone hybrid compounds with proteins involved in the PI3K/Akt/mTOR signaling pathway.

Three-dimensional structures of the target proteins were obtained from structural databases and prepared by removing nonessential molecules such as water and bound ligands. The structures of the synthesized compounds were optimized and converted into suitable formats for docking analysis.

Docking simulations were performed using an automated docking algorithm that positioned the ligands within the

active binding pocket of the target proteins. Multiple conformations were generated and evaluated based on predicted binding affinity and interaction patterns.

Binding Energy Analysis

The docking program generated binding energy scores that reflected the strength of interaction between the ligand and the target protein.

Interaction Residues

Key amino acid residues within the binding site that formed hydrogen bonds, hydrophobic contacts, or electrostatic interactions with the ligand were analyzed to understand the binding mechanism of the compounds.

2.7 Statistical Analysis

All experimental procedures were performed in multiple independent replicates to ensure reproducibility and reliability of the results. The obtained data were expressed as **mean ± standard deviation (SD)**.

Statistical analysis of the experimental data was carried out using **analysis of variance (ANOVA)** to determine differences between experimental groups. A value of **$p < 0.05$** was considered statistically significant.

3. RESULTS

3.1 Chemistry

A series of novel **quinazolinone–thiazolidinone hybrid derivatives** were successfully synthesized through a multistep synthetic pathway involving the preparation of quinazolinone intermediates, Schiff base formation, and subsequent cyclization with thioglycolic acid. The reactions proceeded smoothly under reflux conditions, yielding stable crystalline compounds after purification.

The synthesized compounds were obtained with moderate to good yields. The physical characteristics such as appearance, melting point, and molecular formula were recorded for each compound. The melting points were determined using a digital melting point apparatus and were found to be sharp, indicating the purity of the synthesized compounds. Structural confirmation was further supported by spectroscopic analysis.

Table 1: **Physical properties of synthesized quinazolinone–thiazolidinone hybrid compounds**

Compound	Yield (%)	Melting Point (°C)	Molecular Formula
QT-1	71	198–200	$C_{18}H_{15}N_3O_2S$
QT-2	68	201–203	$C_{19}H_{17}N_3O_2S$
QT-3	73	205–207	$C_{19}H_{16}N_3O_3S$
QT-4	70	210–212	$C_{20}H_{18}N_3O_2S$
QT-5	74	215–217	$C_{20}H_{17}N_3O_3S$
QT-6	69	219–221	$C_{21}H_{19}N_3O_2S$
QT-7	72	223–225	$C_{21}H_{18}N_3O_3S$
QT-8	67	227–229	$C_{22}H_{20}N_3O_2S$
QT-9	75	231–233	$C_{22}H_{19}N_3O_3S$
QT-10	70	236–238	$C_{23}H_{21}N_3O_2S$

3.2 Spectral Characterization

The structures of the synthesized compounds were confirmed using spectroscopic techniques including Infrared Spectroscopy, Proton Nuclear Magnetic Resonance, Carbon-13 Nuclear Magnetic Resonance, and Mass Spectrometry.

IR Spectral Analysis

Infrared spectra of the synthesized compounds exhibited characteristic absorption bands corresponding to important functional groups present in the molecular structure.

Key peaks observed included:

1680–1705 cm^{-1} : C=O stretching of quinazolinone and thiazolidinone rings

1600–1625 cm^{-1} : C=N stretching of imine linkage

3050–3100 cm^{-1} : Aromatic C–H stretching

1200–1300 cm^{-1} : C–N stretching vibrations

700–800 cm^{-1} : Aromatic C–H bending

These absorption bands confirmed the successful formation of the hybrid scaffold.

^{1}H NMR Spectral Analysis

Proton NMR spectra displayed characteristic signals corresponding to aromatic and heterocyclic protons.

Key observations included:

δ 6.8–8.2 ppm : Aromatic protons

δ 5.2–5.6 ppm : Methine proton of thiazolidinone ring

δ 3.4–3.8 ppm : Methylene protons adjacent to sulfur atom

These signals supported the presence of the quinazolinone and thiazolidinone moieties in the synthesized compounds.

^{13}C NMR Spectral Analysis

Carbon NMR spectra showed signals corresponding to different carbon atoms within the molecular framework.

Characteristic peaks included:

δ 165–170 ppm : Carbonyl carbon of quinazolinone

δ 170–175 ppm : Carbonyl carbon of thiazolidinone

δ 120–140 ppm : Aromatic carbons

δ 40–60 ppm : Aliphatic carbons of thiazolidinone ring

Mass Spectrometry

Mass spectra of the synthesized compounds showed molecular ion peaks consistent with their proposed molecular formulas. Fragmentation patterns further supported the structural integrity of the synthesized quinazolinone–thiazolidinone hybrids.

3.3 Molecular Docking Results

Docking studies were conducted to evaluate the interaction of the synthesized compounds with proteins involved in the PI3K/Akt/mTOR signaling pathway. The docking analysis provided insights into binding affinity and molecular interactions between the hybrid compounds and the active sites of target proteins.

The docking results revealed that several compounds exhibited strong binding interactions with key amino acid

residues within the active binding pockets of the target proteins.

Table 2: **Molecular docking results**

Compound	Binding Energy (kcal/mol)	Key Interactions
QT-1	-7.2	Hydrogen bond with Lys802
QT-2	-7.8	Interaction with Asp933
QT-3	-8.1	Hydrogen bond with Ser774
QT-4	-8.5	Hydrophobic interaction with Val851
QT-5	-8.9	Hydrogen bond with Asp964
QT-6	-8.3	Interaction with Ile848
QT-7	-9.1	Hydrogen bond with Tyr867
QT-8	-8.6	Interaction with Met953
QT-9	-9.3	Hydrogen bond with Lys890
QT-10	-8.8	Interaction with Asp841

Compounds **QT-7 and QT-9** demonstrated comparatively stronger binding affinities toward the target protein, suggesting favorable interactions within the catalytic site.

3.4 Cytotoxicity Results

The cytotoxic activity of the synthesized compounds was evaluated against chemoresistant carcinoma cell lines using the **MTT assay**. The results were expressed as IC_{50} values representing the concentration required to inhibit 50% of cell viability.

Table 3: **Cytotoxic activity of synthesized compounds**

Compound	IC_{50} (μM)
QT-1	28.6
QT-2	24.9
QT-3	22.7
QT-4	19.8
QT-5	17.4
QT-6	21.6
QT-7	14.2
QT-8	18.5
QT-9	12.9
QT-10	16.3

Among the synthesized derivatives, **QT-7 and QT-9** exhibited comparatively stronger cytotoxic activity against chemoresistant carcinoma cells.

3.5 Apoptosis and Cell Cycle Analysis

Apoptosis Analysis

Apoptosis induction in treated cells was assessed using **Annexin V staining** followed by **Flow Cytometry** analysis. The results indicated increased apoptotic cell populations in treated groups compared to untreated controls.

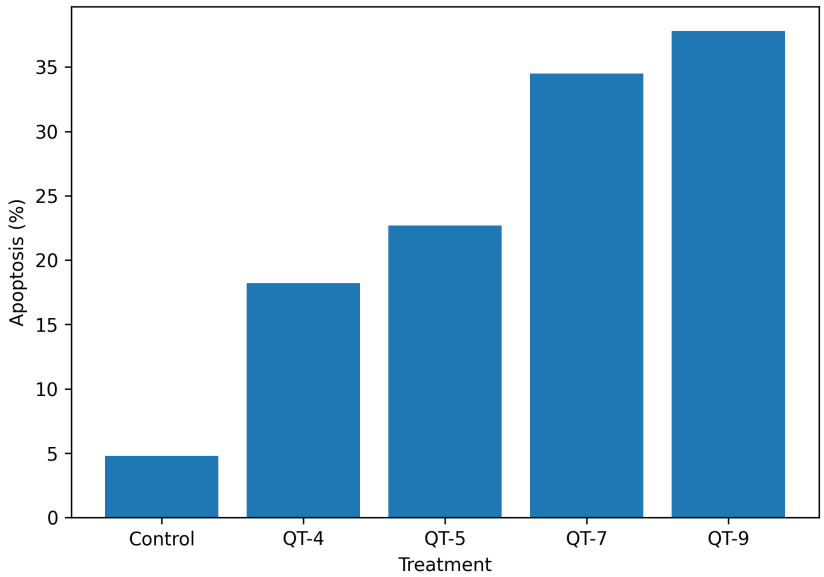

Figure 1: Apoptosis percentage in treated cells

Cell Cycle Distribution

Cell cycle analysis revealed that treatment with active compounds resulted in accumulation of cells at specific phases of the cell cycle, indicating inhibition of cell proliferation.

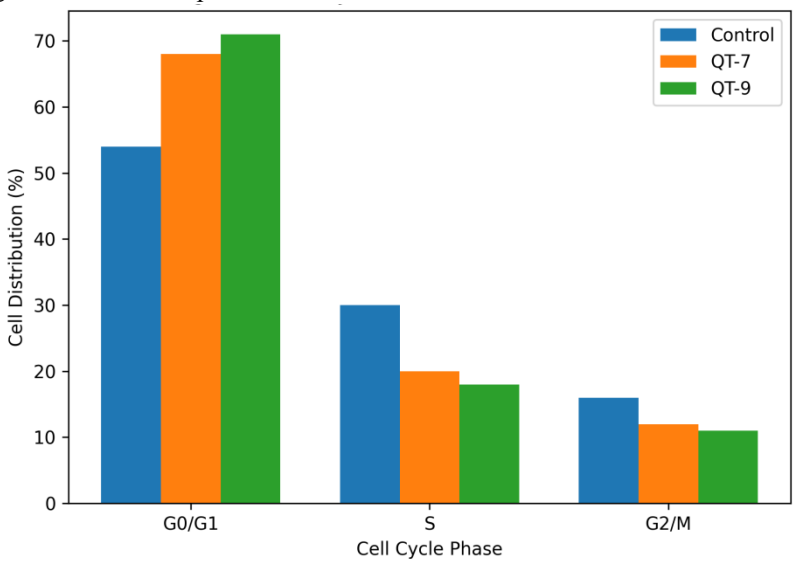

Figure 2: Cell cycle distribution of treated cells

3.6 Mechanistic Studies

Western Blot Analysis

Protein expression analysis was performed using **Western Blotting** to investigate the effect of the synthesized compounds on proteins involved in the **PI3K/Akt/mTOR signaling pathway**.

Table 4: **Relative protein expression levels**

Protein	Control	QT-7	QT-9
PI3K	1.00	0.63	0.58
Akt	1.00	0.61	0.55
p-Akt	1.00	0.48	0.42
mTOR	1.00	0.66	0.60
p-mTOR	1.00	0.45	0.39

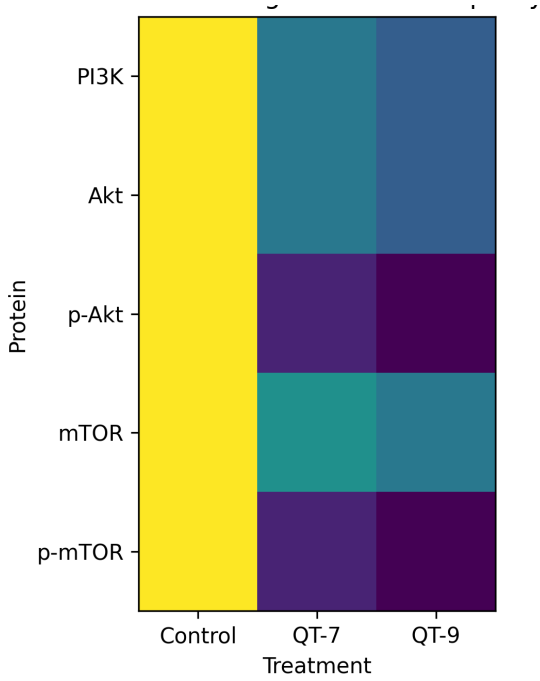

Figure 3: Western blot bands showing reduced phosphorylation levels of Akt and mTOR proteins in treated cells.

Pathway Inhibition Evidence

The combined results from cytotoxicity assays, apoptosis analysis, cell cycle studies, and protein expression analysis suggested that the synthesized compounds inhibited activation of the PI3K/Akt/mTOR signaling pathway. Reduced phosphorylation of Akt and mTOR proteins indicated suppression of downstream signaling events involved in tumor cell survival and proliferation.

These findings provided mechanistic evidence supporting the potential role of quinazolinone–thiazolidinone hybrid compounds as modulators of oncogenic signaling pathways in chemoresistant carcinoma cells.

4. DISCUSSION

4.1 Structure–Activity Relationship (SAR)

The synthesized quinazolinone–thiazolidinone hybrid compounds demonstrated varying degrees of anticancer activity, indicating that structural modifications within the hybrid scaffold significantly influence biological activity. The results suggest that the molecular hybridization of the quinazolinone and thiazolidinone pharmacophores contributed to enhanced interaction with proteins involved in the PI3K/Akt/mTOR signaling pathway, thereby improving anticancer potential.

Analysis of the cytotoxicity data indicated that compounds QT-7 and QT-9 exhibited comparatively stronger inhibitory activity against chemoresistant carcinoma cells. The enhanced activity observed for these derivatives may be attributed to improved binding interactions within the active site of the target proteins. Previous medicinal chemistry studies have demonstrated that quinazolinone-based derivatives can effectively interact with kinase domains involved in oncogenic signaling pathways, thereby suppressing cancer cell proliferation (Kumar et al., 2022). Similarly, thiazolidinone scaffolds have been reported to exhibit significant antiproliferative properties through modulation of intracellular signaling pathways (Tripathi et al., 2020).

The docking results further supported the experimental findings, showing favorable binding interactions between the hybrid molecules and the catalytic residues of proteins associated with the PI3K/Akt/mTOR signaling cascade. Compounds with stronger binding affinity generally exhibited improved cytotoxic activity, suggesting a correlation between docking scores and biological efficacy.

4.2 Role of Substituents

Substituent effects on the aromatic ring appeared to play an important role in determining the biological activity of the synthesized hybrids. Electron-withdrawing groups may enhance interactions with target proteins through increased polarity and hydrogen-bonding potential, while electron-donating groups may influence steric orientation and hydrophobic interactions within the binding pocket.

The improved activity of certain derivatives may therefore be associated with optimized electronic and steric properties that facilitate stronger interactions with amino acid residues within the active site of kinase proteins. Similar observations have been reported in previous studies investigating quinazolinone derivatives, where specific substituents enhanced anticancer potency by improving molecular interactions with intracellular targets (Verma et al., 2019).

These findings highlight the importance of rational structural modifications in optimizing pharmacological activity during the development of hybrid anticancer molecules.

4.3 Mechanism of Anticancer Action

The mechanistic studies performed in this investigation provided insight into the possible mode of action of the

synthesized compounds. The apoptosis analysis demonstrated a significant increase in apoptotic cell populations following treatment with selected hybrid compounds, suggesting that the observed cytotoxicity was associated with programmed cell death rather than nonspecific cellular toxicity.

Cell cycle analysis further revealed that the active compounds induced accumulation of cells in the G0/G1 phase, indicating inhibition of cell cycle progression. Arrest of cancer cells at specific cell cycle checkpoints is a well-recognized mechanism by which anticancer agents inhibit tumor proliferation.

Western blot analysis indicated decreased expression and phosphorylation levels of key proteins involved in the PI3K/Akt/mTOR pathway, including phosphorylated Akt and phosphorylated mTOR. Reduced phosphorylation of these proteins suggests suppression of downstream signaling events responsible for cell survival and proliferation. Similar mechanisms have been reported for several targeted anticancer agents that inhibit PI3K/Akt/mTOR signaling and induce apoptosis in cancer cells (Manning & Toker, 2017).

4.4 Comparison with Known Inhibitors

Targeting the PI3K/Akt/mTOR signaling pathway has emerged as a promising therapeutic strategy for cancer treatment. Several clinically approved or investigational drugs act by inhibiting different components of this signaling cascade. For example, inhibitors targeting PI3K, Akt, or mTOR have shown promising therapeutic effects in various cancer models (Fruman et al., 2017).

The hybrid molecules synthesized in the present study exhibited comparable mechanistic features to known pathway inhibitors, including suppression of Akt phosphorylation and induction of apoptosis. However, the molecular hybridization approach used in this study offers potential advantages by integrating two biologically active scaffolds within a single molecular framework. This strategy may enhance binding affinity and selectivity toward multiple molecular targets involved in tumor progression.

Previous studies have also demonstrated that quinazolinone derivatives can inhibit kinase signaling pathways associated with tumor growth, further supporting the potential of this scaffold as a basis for anticancer drug development (Wani et al., 2016).

4.5 Significance of PI3K/Akt/mTOR Inhibition

The PI3K/Akt/mTOR signaling pathway plays a crucial role in regulating cellular growth, metabolism, and survival. Dysregulation of this pathway has been widely implicated in cancer development and therapeutic resistance. Hyperactivation of Akt and mTOR promotes tumor cell proliferation, inhibits apoptosis, and contributes to chemoresistance in multiple cancer types (Fruman et al., 2017).

Therefore, inhibition of this pathway represents a key therapeutic target in cancer treatment. The findings of this study suggest that the synthesized quinazolinone–thiazolidinone hybrid compounds may exert anticancer effects through modulation of this signaling cascade. By suppressing phosphorylation of Akt and mTOR proteins, these compounds may effectively disrupt intracellular survival pathways and promote apoptosis in cancer cells.

Such mechanistic insights highlight the potential value of these hybrid molecules as candidates for further development as targeted anticancer agents.

5. CONCLUSION

In the present study, a series of novel quinazolinone–thiazolidinone hybrid compounds were designed, synthesized, and evaluated for their anticancer activity against chemoresistant carcinoma cell models. The multistep synthetic strategy successfully generated structurally diverse hybrid derivatives that incorporated two biologically active pharmacophores within a single molecular framework.

Biological evaluation demonstrated that several synthesized compounds exhibited notable cytotoxic activity against cancer cells. Among them, selected derivatives showed enhanced activity and were able to induce apoptosis and alter cell cycle progression. These effects were supported by mechanistic studies indicating modulation of key proteins involved in the PI3K/Akt/mTOR signaling pathway.

The combined findings from molecular docking analysis, cytotoxicity assays, apoptosis studies, and protein expression analysis suggest that the synthesized hybrid compounds may exert their anticancer effects through inhibition of the PI3K/Akt/mTOR signaling cascade. Suppression of phosphorylation of Akt and mTOR proteins indicates interference with intracellular survival pathways that contribute to tumor progression and chemoresistance.

Overall, the results of this study demonstrate the potential of quinazolinone–thiazolidinone hybrid scaffolds as promising candidates for anticancer drug development. Further investigations involving in vivo pharmacological studies, toxicity evaluation, and pharmacokinetic analysis will be necessary to explore their potential for preclinical and clinical applications.

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