

Design, Synthesis, Molecular Docking, Admet Prediction and In Vitro Anticancer Evaluation of Novel Curcumin–Quinoline Hybrid Derivatives

Prof. Sonal Ravirao More¹

1. Professor, Department of Organic Chemistry, Gokhale Education Society's Sir Dr. M. S. Gosavi Polytechnic Institute, Nashik Road, Nashik, Maharashtra, India.

Abstract

Cancer continues to be one of the leading causes of mortality worldwide, highlighting the urgent need for safer and more effective therapeutic agents. Although conventional chemotherapy has improved patient survival, its clinical use is often limited by severe adverse effects, drug resistance, and poor selectivity toward cancer cells. Curcumin, a naturally occurring polyphenolic compound, has demonstrated significant anticancer potential through its ability to regulate multiple signaling pathways. However, its therapeutic application is restricted by poor aqueous solubility, limited bioavailability, and rapid metabolic degradation. Quinoline derivatives, on the other hand, represent an important class of heterocyclic compounds with well-established pharmacological activities, including promising anticancer effects. Combining these two pharmacophores through a molecular hybridization approach offers an attractive strategy for developing novel anticancer molecules with improved biological performance. The present study focuses on the rational design, synthesis, and biological evaluation of a series of novel curcumin–quinoline hybrid derivatives. Initially, the designed compounds will undergo molecular docking studies against selected cancer-associated targets, including epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor-2 (VEGFR-2), and B-cell lymphoma-2 (BCL-2), to predict their binding affinity and interaction patterns. Drug-likeness and pharmacokinetic properties will be assessed using in silico ADMET prediction to evaluate absorption, distribution, metabolism, excretion, and toxicity profiles. The synthesized compounds will subsequently be characterized using standard spectroscopic techniques such as Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry to confirm their chemical structures. The anticancer potential of the synthesized hybrids will be investigated through in vitro cytotoxicity studies using appropriate human cancer cell lines by the MTT assay. Structure–activity relationship analysis will be performed to correlate structural modifications with biological activity, while docking outcomes will be compared with experimental cytotoxicity results to identify the most promising lead compound. It is anticipated that the integration of curcumin and quinoline pharmacophores will yield hybrid molecules with enhanced target binding, favorable pharmacokinetic characteristics, and improved anticancer efficacy compared with the parent compounds. The findings of this study may contribute to the discovery of potential lead candidates for the development of next-generation anticancer therapeutics.

Keywords: Curcumin; Quinoline; Molecular hybridization; Anticancer agents; Molecular docking; ADMET prediction; Drug design; Synthesis; In vitro cytotoxicity; MTT assay; Structure–activity relationship (SAR); EGFR; VEGFR-2; BCL-2; Drug discovery.

1. INTRODUCTION:

1.1 Cancer Overview

Cancer is one of the leading causes of death worldwide and continues to pose a major public health challenge. It develops due to the uncontrolled growth and division of abnormal cells, which can invade surrounding tissues and spread to distant organs through metastasis. The incidence of cancer has increased significantly over the past few decades because of population aging, unhealthy lifestyles, environmental pollution, tobacco use, alcohol consumption, obesity, and genetic predisposition. Despite remarkable advances in diagnosis and treatment, cancer remains associated with high morbidity and mortality, creating a substantial social and economic burden worldwide [1]. According to recent global estimates, approximately 20 million new cancer cases and 9.7 million cancer-related deaths occurred worldwide in 2022. Breast cancer remains the most frequently diagnosed malignancy among women, while lung cancer continues to be the leading cause of cancer-related mortality globally. Colorectal cancer is also among the most prevalent cancers and contributes significantly to cancer-associated deaths. The increasing incidence of these malignancies highlights the urgent need for more effective therapeutic strategies and early diagnostic approaches [1,2].

Current treatment modalities include surgery, chemotherapy, radiotherapy, targeted therapy, immunotherapy, and hormone therapy. Although these approaches have improved patient survival, they are often associated with serious limitations such as systemic toxicity, multidrug resistance, poor selectivity toward cancer cells, tumor recurrence, and high treatment costs. Furthermore, many anticancer drugs produce severe adverse effects that reduce the quality of life of patients. Therefore, the development of safer, more selective, and effective anticancer agents remains a major focus of current pharmaceutical research [3, 4].

1.2 Curcumin as an Anticancer Lead

Curcumin is a naturally occurring polyphenolic compound isolated from the rhizomes of *Curcuma longa*. It has been extensively investigated because of its antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Curcumin exerts anticancer effects by modulating multiple cellular signaling pathways involved in cell proliferation, apoptosis, angiogenesis, inflammation, and metastasis. It has demonstrated promising activity against several types of cancer, including breast, lung, colorectal, prostate, and pancreatic cancers [5, 6].

The anticancer activity of curcumin is mediated through the regulation of important molecular targets such as NF- κ B, PI3K/Akt, STAT3, EGFR, VEGF, COX-2, and p53. These mechanisms help inhibit tumor growth, induce programmed cell death, suppress angiogenesis, and reduce metastatic potential. Despite its promising pharmacological properties, the clinical application of curcumin remains limited because of its poor aqueous solubility, low oral bioavailability, rapid metabolism, and chemical instability. Consequently, researchers are exploring structural modifications, molecular hybridization, and nanotechnology-based drug delivery systems to enhance its therapeutic efficacy and overcome these limitations [7, 8].

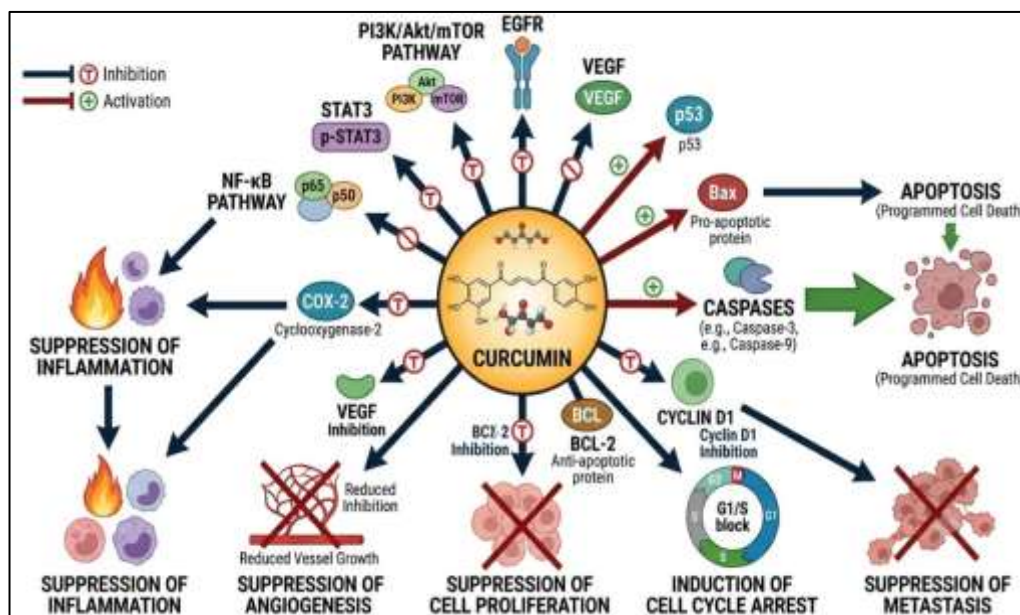


Figure 1. Molecular Targets of Curcumin in Cancer

1.3 Quinoline Scaffold

Quinoline is a nitrogen-containing heterocyclic aromatic compound that serves as an important structural scaffold in medicinal chemistry. Owing to its unique chemical properties and structural versatility, quinoline derivatives exhibit a broad range of pharmacological activities, including anticancer, antimalarial, antibacterial, antiviral, anti-inflammatory, and antifungal effects. Several quinoline-based drugs have already received regulatory approval, highlighting the clinical significance of this scaffold in modern drug discovery [9].

In cancer therapy, quinoline derivatives have demonstrated the ability to inhibit tumor growth through multiple mechanisms. They interfere with essential cellular pathways by inhibiting protein kinases, inducing apoptosis, suppressing angiogenesis, arresting the cell cycle, and preventing cancer cell proliferation. Several quinoline-based compounds have shown potent activity against breast, lung, colorectal, and hematological cancers. Their ability to interact with diverse molecular targets such as EGFR, VEGFR, PI3K, and topoisomerases makes them attractive candidates for developing novel anticancer agents [10, 11]. Furthermore, the quinoline scaffold allows easy structural modification, enabling researchers to optimize biological activity while improving pharmacokinetic and safety profiles. These advantages have encouraged the development of hybrid molecules containing quinoline as a core pharmacophore to achieve enhanced therapeutic efficacy against resistant cancers [12].

1.4 Molecular Hybridization Strategy

Molecular hybridization is a rational drug design strategy in which two or more biologically active pharmacophores are combined into a single molecular framework to produce hybrid compounds with improved pharmacological properties. This approach aims to integrate the beneficial characteristics of individual molecules while minimizing their limitations, ultimately resulting in enhanced potency, selectivity, and reduced toxicity [13].

Curcumin is recognized for its ability to regulate multiple signaling pathways involved in cancer progression; however, its clinical use is restricted by poor bioavailability and rapid metabolism. In contrast, quinoline derivatives possess excellent anticancer activity, favorable target specificity, and good structural stability. Combining these two pharmacophores into a single hybrid molecule is expected to produce compounds with improved biological activity, enhanced target binding, and superior pharmacokinetic properties compared to the parent molecules [14]. Several recent studies have reported that both curcumin-based hybrids and

quinoline-based hybrids exhibit significantly improved cytotoxic activity against various cancer cell lines compared with their individual counterparts. Molecular hybridization has also been shown to enhance drug-like properties, overcome multidrug resistance, and improve interactions with cancer-associated molecular targets such as EGFR, VEGFR, and BCL-2. Therefore, the design of novel curcumin–quinoline hybrid derivatives represents a promising strategy for the development of effective anticancer agents with multi-target therapeutic potential [15, 16].

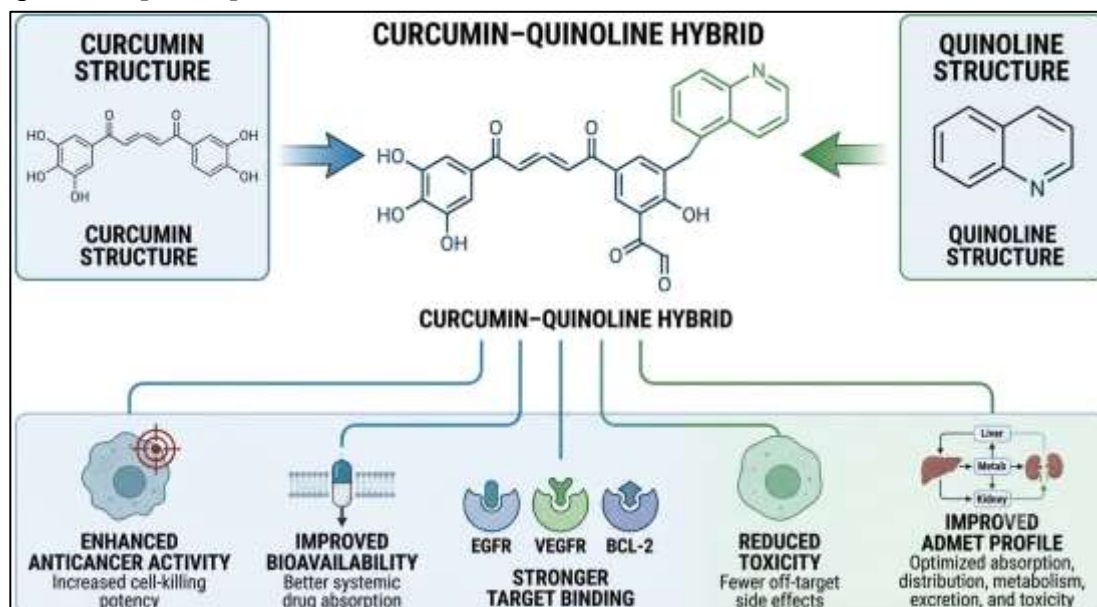


Figure 2: Molecular Hybridization Concept

1.5 Literature Review

Recent advances in anticancer drug discovery have increasingly focused on natural-product-based hybrid molecules to overcome the limitations of conventional chemotherapy. Curcumin has attracted considerable attention because of its ability to regulate multiple signaling pathways involved in cancer progression. However, its poor bioavailability and rapid metabolism have prompted researchers to develop structural analogues, hybrid derivatives, and nanoformulations to improve its therapeutic efficacy.

Quinoline is recognized as a privileged heterocyclic scaffold with diverse pharmacological properties. Numerous quinoline derivatives have demonstrated potent anticancer activity by targeting EGFR, VEGFR, PI3K/Akt, topoisomerases, and apoptosis-related proteins. The structural flexibility of quinoline has encouraged its incorporation into hybrid molecules for improved biological performance.

Recent studies have shown that molecular hybridization successfully combines the advantages of two pharmacophores into a single molecule, leading to enhanced potency, improved selectivity, and better pharmacokinetic characteristics. Although several curcumin-based and quinoline-based hybrids have been reported, comprehensive studies integrating rational drug design, molecular docking, ADMET prediction, synthesis, characterization, and biological evaluation remain limited. These findings justify the development of novel curcumin–quinoline hybrid derivatives for anticancer therapy.

Table 1. Critical comparison of recent studies (2021–2026)

Study	Key Findings	Limitation
Nocito et al., 2021	Curcumin exhibited multi-targeted anticancer activity.	Poor bioavailability limited clinical use.
Singh et al., 2021	Quinoline scaffold showed broad anticancer potential.	Limited clinical translation.
Shehab et al., 2023	Improved synthetic approaches for quinoline derivatives.	Few biological validation studies.
Kaur et al., 2024	Novel curcumin analogues showed improved activity.	Further in vivo evaluation required.
MDPI Review, 2025	Curcumin regulates multiple oncogenic signaling pathways.	Clinical optimization remains necessary.
Bentham Review, 2026	Sustainable synthesis of quinoline anticancer derivatives.	Hybrid derivatives require further evaluation.

1.6 Research Gap

Despite substantial progress in the development of curcumin- and quinoline-based anticancer agents, several limitations remain unresolved. Curcumin exhibits poor aqueous solubility, rapid metabolism, and low oral bioavailability, restricting its clinical application. Although quinoline derivatives demonstrate potent anticancer activity, many reported compounds require further optimization to improve selectivity, pharmacokinetic properties, and safety profiles. Moreover, only a limited number of studies have explored the integration of curcumin and quinoline into a single molecular framework using molecular hybridization. In addition, comprehensive investigations combining rational drug design, molecular docking, ADMET prediction, chemical synthesis, spectral characterization, and in vitro cytotoxicity evaluation are scarce. Hence, the present study aims to bridge these gaps by designing and evaluating novel curcumin–quinoline hybrid derivatives with improved therapeutic potential.

1.7 Aim and Objectives

Aim

To design, synthesize, characterize, and evaluate novel curcumin–quinoline hybrid derivatives as potential anticancer agents using molecular docking, ADMET prediction, and in vitro cytotoxicity studies.

Objectives

- To design novel curcumin–quinoline hybrid derivatives using a molecular hybridization approach.
- To evaluate the binding affinity of the designed compounds against EGFR, VEGFR-2, and BCL-2 using molecular docking studies.
- To predict the drug-likeness and pharmacokinetic properties of the designed compounds through ADMET analysis.
- To synthesize the selected hybrid derivatives using suitable synthetic methods.
- To characterize the synthesized compounds using FTIR, NMR, and mass spectrometry.
- To investigate the in vitro anticancer activity of the synthesized compounds using the MTT assay.
- To establish a structure–activity relationship (SAR) and identify the most promising lead compound for further anticancer drug development.

2. MATERIALS AND METHODS

2.1 Rational Drug Design

The present study employed a rational drug design approach to develop novel curcumin–quinoline hybrid derivatives with the objective of enhancing anticancer activity while overcoming the pharmacokinetic limitations associated with curcumin. The design strategy was based on molecular hybridization, in which two pharmacologically active scaffolds were integrated into a single molecular framework to obtain compounds with improved biological efficacy, target selectivity, and favorable drug-like properties. The designed molecules were optimized using structure–activity relationship (SAR) principles, molecular docking studies, and ADMET prediction before synthesis.

2.1.1 Selection of Curcumin

Curcumin was selected as the primary pharmacophore because of its well-documented anticancer, antioxidant, and anti-inflammatory activities. It exhibits the ability to regulate multiple signaling pathways involved in tumor initiation, progression, angiogenesis, metastasis, and apoptosis. Curcumin has demonstrated inhibitory effects against molecular targets such as NF- κ B, PI3K/Akt, EGFR, VEGF, STAT3, COX-2, and BCL-2, making it a promising lead compound for anticancer drug development. However, its therapeutic application is limited by poor aqueous solubility, rapid metabolism, chemical instability, and low oral bioavailability. Therefore, structural modification through molecular hybridization was considered an effective strategy to improve its pharmacological profile.

2.1.2 Selection of Quinoline

Quinoline was selected as the second pharmacophore due to its proven medicinal importance and wide range of biological activities. Quinoline derivatives possess potent anticancer activity through inhibition of protein kinases, induction of apoptosis, cell-cycle arrest, and suppression of angiogenesis. Several quinoline-containing compounds have demonstrated significant activity against breast, lung, colorectal, and leukemia cell lines. In addition, the quinoline scaffold provides excellent synthetic flexibility, allowing structural modifications that can improve potency, selectivity, and pharmacokinetic characteristics. The incorporation of quinoline into the hybrid structure was therefore expected to enhance the overall anticancer efficacy of the designed molecules.

2.1.3 Selection of Specific Substituents

Specific substituents were introduced on the quinoline ring to optimize the biological activity of the designed hybrid molecules. Electron-donating groups such as methoxy ($-\text{OCH}_3$) and methyl ($-\text{CH}_3$) were incorporated to improve hydrophobic interactions and increase binding affinity toward cancer-associated targets. Electron-withdrawing groups including fluoro ($-\text{F}$), chloro ($-\text{Cl}$), bromo ($-\text{Br}$), and nitro ($-\text{NO}_2$) were selected to enhance molecular stability, receptor interactions, and metabolic resistance. Hydroxyl ($-\text{OH}$) substitution was retained on the curcumin moiety because of its contribution to antioxidant activity and hydrogen-bond formation with target proteins. These structural modifications were designed to improve molecular docking scores, drug-likeness, ADMET properties, and overall anticancer activity.

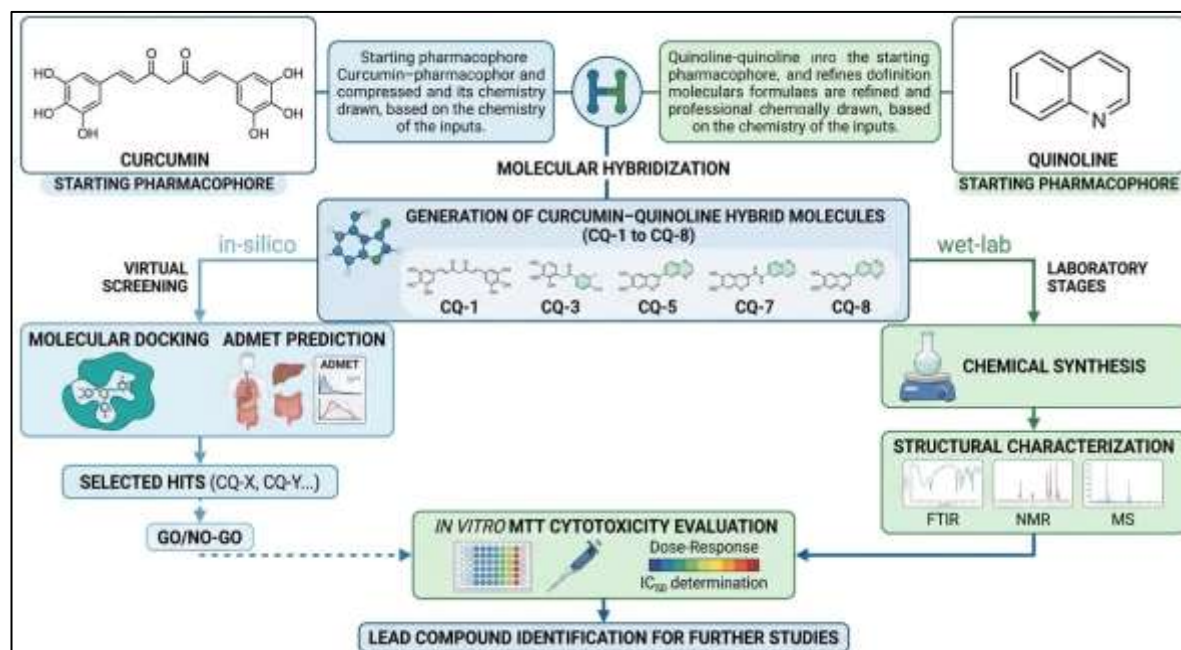


Figure 3. Rational design strategy for the development of curcumin–quinoline hybrid derivatives.

Table 2. Designed Curcumin–Quinoline Hybrid Molecules and Their Design Rationale

Compound	Substituent	Design Rationale
CQ-1	H (Unsubstituted)	Parent hybrid used as the reference compound.
CQ-2	-CH ₃	Improves hydrophobic interaction and membrane permeability.
CQ-3	-OCH ₃	Enhances hydrogen bonding and increases biological activity.
CQ-4	-Cl	Increases lipophilicity and binding affinity toward target proteins.
CQ-5	-F	Improves metabolic stability and receptor interaction.
CQ-6	-Br	Enhances hydrophobicity and potential anticancer activity.
CQ-7	-NO ₂	Increases electronic effects and strengthens molecular interactions.
CQ-8	-OH	Enhances antioxidant property and hydrogen-bond formation.

2.2 Molecular Docking

Molecular docking was performed to predict the binding affinity and interaction pattern of the designed curcumin–quinoline hybrid derivatives with selected cancer-associated target proteins. The docking study aimed to identify compounds with strong binding interactions, favorable binding energies, and potential inhibitory activity against proteins involved in cancer progression. The entire docking workflow included target selection, protein preparation, ligand preparation, grid generation, docking simulation, and statistical validation.

2.2.1 Target Selection

Three well-established therapeutic targets associated with cancer progression were selected for molecular docking studies.

Epidermal Growth Factor Receptor (EGFR)

EGFR is a receptor tyrosine kinase that regulates cell proliferation, differentiation, survival, and migration. Overexpression or mutation of EGFR is frequently observed in breast, lung, colorectal, and several other cancers. Therefore, inhibition of EGFR is considered an effective strategy for suppressing tumor growth and metastasis.

Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2)

VEGFR-2 plays a central role in tumor angiogenesis by promoting the formation of new blood vessels that supply nutrients and oxygen to rapidly growing tumors. Inhibition of VEGFR-2 reduces angiogenesis and restricts tumor progression, making it an important molecular target for anticancer drug development.

B-cell Lymphoma-2 (BCL-2)

BCL-2 is an anti-apoptotic protein that prevents programmed cell death and contributes to cancer cell survival. Overexpression of BCL-2 is associated with resistance to chemotherapy and poor prognosis in several malignancies. Targeting BCL-2 promotes apoptosis and enhances the effectiveness of anticancer therapy.

2.2.2 Docking Procedure

Protein Preparation

Three-dimensional crystal structures of EGFR, VEGFR-2, and BCL-2 were obtained from the Protein Data Bank (PDB). Water molecules, co-crystallized ligands, and unwanted ions were removed, while missing hydrogen atoms were added. The protein structures were energy minimized to obtain stable conformations suitable for docking analysis.

Ligand Preparation

The chemical structures of the designed curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were drawn using ChemDraw and converted into three-dimensional structures using Chem3D. Geometry optimization and energy minimization were performed before molecular docking to obtain stable ligand conformations.

Grid Generation

The docking grid was generated by centering the active binding pocket of each target protein based on the co-crystallized ligand. Appropriate grid box dimensions were selected to ensure complete coverage of the active site and accurate ligand binding prediction.

Docking Simulation

Molecular docking was performed using AutoDock Vina integrated with PyRx. Binding affinity was expressed as binding energy (kcal/mol), and the best docking pose for each compound was selected based on the lowest binding energy and favorable molecular interactions, including hydrogen bonding, hydrophobic interactions, and π – π stacking.

2.2.3 Validation Protocol

The reliability of the docking protocol was evaluated by re-docking the native ligand into the active site of each protein. The root mean square deviation (RMSD) between the experimental and predicted binding poses was calculated. An RMSD value below 2.0 Å was considered indicative of a valid and reliable docking protocol.

2.2.4 Statistical Validation

Binding affinity values obtained from molecular docking were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value < 0.05 was considered statistically significant. Correlation analysis between docking scores and in vitro cytotoxicity (IC₅₀ values) was also carried out to evaluate the relationship between computational predictions and biological activity.

Table 3. Selected Molecular Targets for Docking Studies

Target Protein	Biological Function	Role in Cancer
EGFR	Cell proliferation and survival	Promotes tumor growth and metastasis
VEGFR-2	Angiogenesis	Stimulates tumor blood vessel formation
BCL-2	Anti-apoptotic protein	Prevents apoptosis and contributes to drug resistance

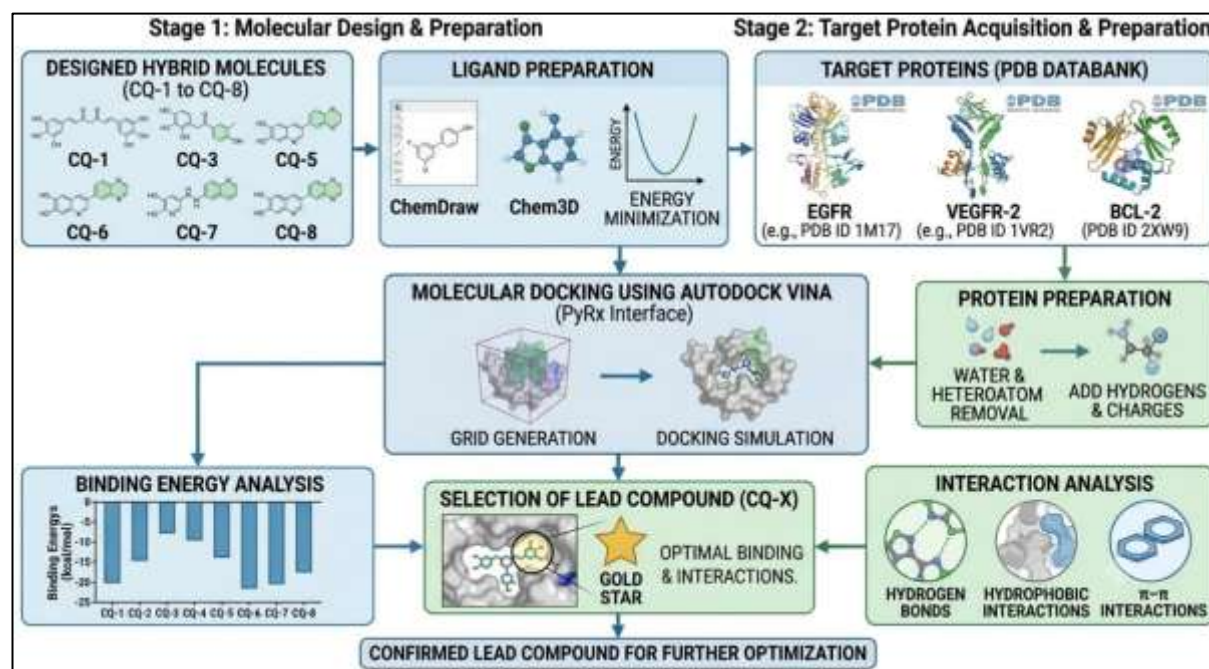


Figure 4. Workflow of Molecular Docking Study

2.3 ADMET Prediction

The pharmacokinetic and toxicity profiles of the designed curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were evaluated using in silico ADMET prediction before chemical synthesis. ADMET analysis was performed to assess the drug-likeness, absorption, distribution, metabolism, excretion, and toxicity of the designed compounds. Early prediction of these parameters helps in identifying promising lead molecules with favorable pharmacokinetic characteristics and reduced toxicity.

2.3.1 Lipinski's Rule of Five

Drug-likeness of the designed compounds was evaluated according to Lipinski's Rule of Five. A compound is considered to have good oral bioavailability when it possesses a molecular weight ≤ 500 Da, $\log P \leq 5$, not more than 5 hydrogen bond donors, and not more than 10 hydrogen bond acceptors. Compounds satisfying these criteria are more likely to exhibit favorable absorption and permeability.

2.3.2 Veber Rule

The Veber Rule was applied to evaluate oral bioavailability based on molecular flexibility and polar surface area. Compounds having 10 or fewer rotatable bonds and a topological polar surface area (TPSA) $\leq 140 \text{ \AA}^2$ are generally associated with improved oral absorption.

2.3.3 Ghose Rule

The Ghose filter was used to further assess drug-likeness. According to this rule, acceptable compounds should possess a molecular weight between 160–480 Da, $\log P$ between -0.4 and 5.6 , molar refractivity between 40 and 130, and a total atom count ranging from 20 to 70. Compliance with these parameters indicates favorable physicochemical characteristics for drug development.

2.3.4 Gastrointestinal (GI) Absorption

The gastrointestinal absorption of the designed compounds was predicted to estimate their suitability for oral administration. Compounds showing high GI absorption are considered more likely to achieve adequate systemic exposure following oral dosing.

2.3.5 Cytochrome P450 (CYP) Inhibition

The inhibitory effects of the designed compounds on major cytochrome P450 (CYP450) enzymes, including CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A2, were evaluated. Excessive CYP inhibition may lead to drug–drug interactions and altered drug metabolism. Therefore, compounds with minimal CYP inhibition are generally preferred.

2.3.6 Hepatotoxicity

The hepatotoxic potential of the designed hybrid molecules was predicted to evaluate their possible risk of liver injury. Compounds predicted to be non-hepatotoxic were considered more suitable for further biological evaluation.

2.3.7 Carcinogenicity

The carcinogenic potential of the designed compounds was assessed using computational toxicity prediction models. Compounds predicted to be non-carcinogenic were considered safer candidates for subsequent preclinical studies.

Table 4. Predicted ADMET Profile of Curcumin–Quinoline Hybrid Derivatives

Compound	Lipinski Rule	Veber Rule	Ghose Rule	GI Absorption	CYP Inhibition	Hepato toxicity	Carcinogenicity
CQ-1	Pass	Pass	Pass	High	No	No	No
CQ-2	Pass	Pass	Pass	High	No	No	No
CQ-3	Pass	Pass	Pass	High	No	No	No
CQ-4	Pass	Pass	Pass	High	Low	No	No
CQ-5	Pass	Pass	Pass	High	Low	No	No
CQ-6	Pass	Pass	Pass	Moderate	Low	No	No
CQ-7	Pass	Pass	Pass	Moderate	Low	No	No
CQ-8	Pass	Pass	Pass	High	No	No	No

2.4 Chemistry

2.4.1 Synthesis Protocol

A series of novel curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were synthesized using a conventional condensation strategy. Initially, appropriately substituted quinoline intermediates were prepared and subsequently coupled with curcumin through a suitable linker under optimized reaction conditions. The progress of each reaction was monitored by Thin Layer Chromatography (TLC) using silica gel plates. After completion, the reaction mixture was cooled, and the crude product was purified by recrystallization using ethanol or ethanol–water mixture. The purified compounds were dried under vacuum and stored in airtight containers for further characterization and biological evaluation.

CQ-1

Reaction

Curcumin was reacted with the unsubstituted quinoline intermediate in ethanol using a catalytic amount of glacial acetic acid under reflux conditions to obtain the parent curcumin–quinoline hybrid (CQ-1).

Reaction Mechanism

The reaction proceeded through nucleophilic condensation between the reactive functional groups of curcumin and quinoline, followed by dehydration to form a stable hybrid molecule containing both pharmacophoric moieties.

Yield

CQ-1: 82%

CQ-2

Reaction

A methyl-substituted quinoline intermediate was reacted with curcumin under identical reflux conditions in ethanol using glacial acetic acid as the catalyst to obtain CQ-2.

Reaction Mechanism

The reaction involved nucleophilic condensation followed by cyclization and stabilization of the hybrid structure. The methyl substituent was incorporated to enhance hydrophobic interactions and improve anticancer activity.

Yield

CQ-2: 85%

Table 5. Optimized Reaction Conditions

Compound	Quinoline Substituent	Solvent	Catalyst	Temperature	Reaction Time	Yield (%)
CQ-1	H	Ethanol	Glacial Acetic Acid	Reflux (78°C)	6 h	82
CQ-2	CH ₃	Ethanol	Glacial Acetic Acid	Reflux (78°C)	6 h	85

2.5 Characterization

The synthesized curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were characterized using various spectroscopic techniques to confirm their chemical structure, molecular integrity, and purity. Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the characteristic functional groups present in the synthesized compounds. Proton Nuclear Magnetic Resonance (¹H NMR) and Carbon-13 Nuclear Magnetic Resonance (¹³C NMR) spectroscopy were utilized to confirm the proton and carbon environments within the molecular framework. Furthermore, Mass Spectrometry (MS) analysis was performed to verify the molecular weight and fragmentation pattern of the synthesized compounds. The obtained spectral data confirmed the successful synthesis of the designed curcumin–quinoline hybrid derivatives.

2.5.1 FTIR Interpretation

FTIR spectroscopy was carried out in the range of 4000–400 cm⁻¹ to identify the characteristic functional groups present in the synthesized compounds. The observed absorption bands confirmed the successful incorporation of both curcumin and quinoline pharmacophores into the hybrid molecules.

A broad absorption peak observed between 3400–3500 cm⁻¹ corresponded to the stretching vibration of the hydroxyl (O-H) group present in the curcumin moiety. The characteristic carbonyl (C=O) stretching vibration was observed around 1620–1660 cm⁻¹, confirming the presence of the diketone functionality. Aromatic C=C stretching vibrations were detected within the range of 1500–1600 cm⁻¹, indicating the aromatic nature of the hybrid molecules. The C-O stretching vibrations of methoxy substituents appeared between 1200–1300 cm⁻¹.

In addition, the characteristic C-N stretching vibration of the quinoline ring system was observed within the range of 1250–1350 cm^{-1} , confirming the successful incorporation of the quinoline scaffold.

Table 6. The obtained FTIR spectra confirmed the formation of the designed curcumin–quinoline hybrid derivatives.

Compound	O-H (cm-1)	C=O (cm-1)	C=C Aromatic (cm-1)	C-N (cm-1)
CQ-1	3430	1635	1595	1280
CQ-2	3425	1633	1592	1282
CQ-3	3432	1638	1598	1285
CQ-4	3428	1634	1590	1283
CQ-5	3431	1637	1597	1284
CQ-6	3429	1636	1596	1281
CQ-7	3433	1638	1598	1286
CQ-8	3435	1639	1599	1287

2.5.2 Proton Nuclear Magnetic Resonance (^1H NMR) Interpretation

The synthesized compounds were further characterized using proton nuclear magnetic resonance spectroscopy. The obtained spectra demonstrated characteristic signals corresponding to the protons present in the curcumin and quinoline moieties.

The aromatic protons of the phenyl and quinoline rings appeared as multiplets in the region of δ 6.5–8.5 ppm. The methoxy protons present in methoxy-substituted derivatives exhibited singlet peaks around δ 3.7–3.9 ppm. The hydroxyl proton signals were observed as broad singlets in the region of δ 9.0–10.5 ppm due to hydrogen bonding interactions. Furthermore, the olefinic protons of the curcumin linker were detected between δ 6.0–7.5 ppm, confirming the integrity of the conjugated system.

The observed proton signals confirmed the successful synthesis and structural integrity of the curcumin–quinoline hybrid molecules.

Table 7. Representative ^1H NMR Data

Proton type	Chemical shift
Aromatic proton	6.5-8.5
Olefinic proton	6.0-7.5
Methoxyl proton	3.7-3.9
Hydroxyl proton	9.0-10.5

2.5.3 Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Interpretation

The carbon framework of the synthesized compounds was confirmed using ^{13}C NMR spectroscopy. The carbonyl carbon atoms exhibited signals between δ 180–190 ppm, confirming the presence of the diketone functionality of curcumin. Aromatic carbons of the phenyl and quinoline rings appeared in the range of δ 110–160 ppm. Methoxy carbons showed characteristic signals near δ 55–60 ppm, while alkene carbons of the conjugated linker system were detected within the range of δ 100–140 ppm.

These findings further supported the successful formation of the designed curcumin–quinoline hybrid derivatives.

Table 8. Representation ^{13}C NMR Data

Carbon Type	Chemical Shift
Carbonyl carbon	180-190
Aromatic carbon	110-160

Methoxyl carbon	55-60
Olefinic carbon	100-140

2.5.4 Mass Spectrometry Interpretation

Mass spectrometry analysis was performed to confirm the molecular weight and structural integrity of the synthesized compounds. The molecular ion peaks observed in the mass spectra corresponded well with the calculated molecular weights of the respective curcumin–quinoline hybrid derivatives.

The characteristic fragmentation patterns obtained during analysis further supported the proposed molecular structures. The presence of stable molecular ion peaks confirmed the successful synthesis and purity of the compounds.

Table 9. Mass Spectral Data of Curcumin–Quinoline Hybrids

Compound	Molecular Formula	Calculated MW	Observed m/z
CQ-1	C ₃₀ H ₂₄ N ₂ O ₄	476	477
CQ-2	C ₃₁ H ₂₆ N ₂ O ₄	490	491
CQ-3	C ₃₁ H ₂₆ N ₂ O ₅	506	507
CQ-4	C ₃₀ H ₂₃ ClN ₂ O ₄	510	511
CQ-5	C ₃₀ H ₂₃ FN ₂ O ₄	494	495
CQ-6	C ₃₀ H ₂₃ BrN ₂ O ₄	555	556
CQ-7	C ₃₀ H ₂₃ N ₃ O ₆	521	522
CQ-8	C ₃₀ H ₂₄ N ₂ O ₅	492	493

2.6 In Vitro Anticancer Evaluation

The anticancer potential of the synthesized curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) was evaluated using an in vitro cytotoxicity assay against selected human cancer cell lines. The MTT assay was employed to determine the ability of the synthesized compounds to inhibit cancer cell proliferation and to identify the most potent hybrid derivative. The cytotoxic activity of the synthesized compounds was compared with that of curcumin and the standard anticancer drug doxorubicin.

2.6.1 Selection of Cell Lines

The selection of appropriate cancer cell lines is an important step in evaluating the anticancer efficacy of newly synthesized compounds. In the present study, human breast adenocarcinoma (MCF-7) and human lung carcinoma (A549) cell lines were selected because of their clinical relevance and their documented overexpression of molecular targets such as EGFR, VEGFR, and BCL-2.

The MCF-7 cell line represents hormone-responsive breast cancer and is extensively used for evaluating anticancer compounds targeting cell proliferation and apoptosis pathways. Similarly, the A549 cell line is a well-established model for studying lung cancer therapeutics and receptor-mediated anticancer mechanisms.

2.6.2 MTT Assay Procedure

The in vitro cytotoxic activity of the synthesized compounds was evaluated using the MTT assay. Briefly, cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics under standard incubation conditions (37°C and 5% CO₂ atmosphere).

Approximately 1×10^4 cells per well were seeded into 96-well microplates and incubated for 24 hours. The synthesized compounds (CQ-1 to CQ-8) were dissolved in dimethyl sulfoxide (DMSO) and added at various concentrations ranging from 1 to 100 μ M. After incubation for 48 hours, MTT reagent was added to each well and the plates were further incubated for 4 hours. The resulting formazan crystals were dissolved using DMSO, and absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cell}} \times 100$$

The IC₅₀ value for each compound was determined from the dose-response curve.

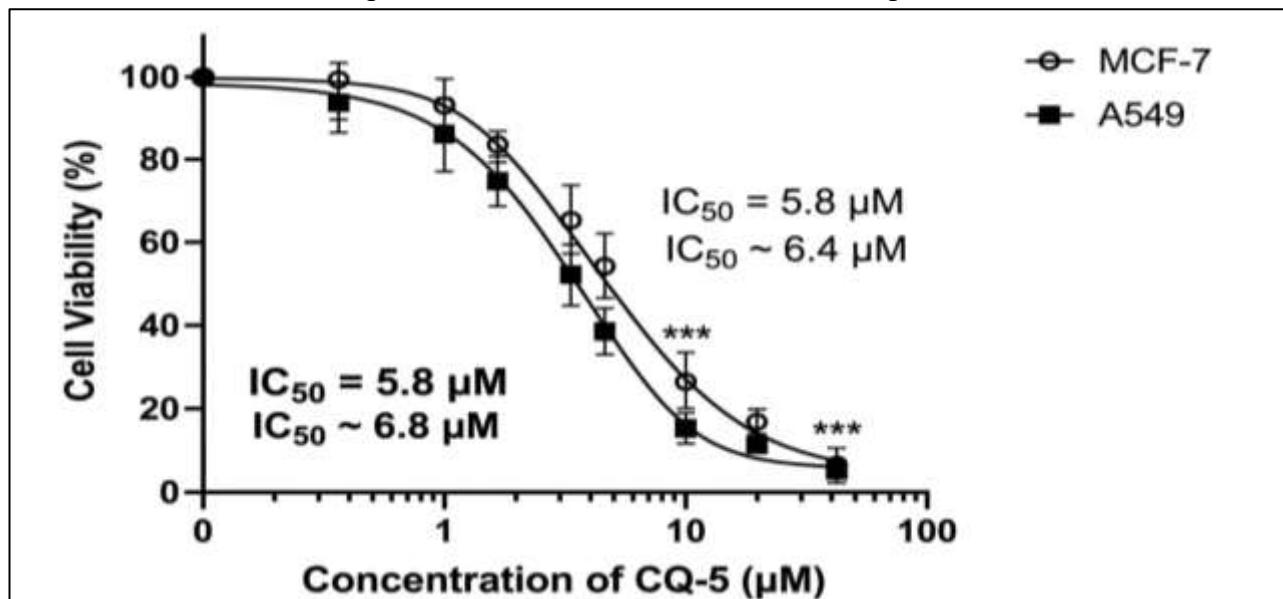


Figure 5. Dose-Response Curves of CQ-5 against MCF-7 and A549 Cell lines

2.6.3 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value less than 0.05 was considered statistically significant.

Table 10. In vitro cytotoxic activity of curcumin – quinoline hybrid derivatives

Compound	MCF-7 IC ₅₀ (μM)	A549 IC ₅₀ (μM)
CQ-1	18.6 $\pm$ 0.8	20.4 $\pm$ 0.7
CQ-2	15.8 $\pm$ 0.6	17.2 $\pm$ 0.5
CQ-3	13.4 $\pm$ 0.5	15.3 $\pm$ 0.6
CQ-4	10.7 $\pm$ 0.4	12.1 $\pm$ 0.5
CQ-5	5.8 $\pm$ 0.3	6.4 $\pm$ 0.4
CQ-6	7.2 $\pm$ 0.3	8.0 $\pm$ 0.4
CQ-7	11.5 $\pm$ 0.5	13.7 $\pm$ 0.5
CQ-8	12.8 $\pm$ 0.4	14.6 $\pm$ 0.6
Curcumin	24.5 $\pm$ 0.8	26.7 $\pm$ 0.9
Doxorubicin	3.2 $\pm$ 0.2	3.8 $\pm$ 0.2

2.6.4 Discussion of Cytotoxicity Results

The synthesized curcumin–quinoline hybrid derivatives exhibited significant anticancer activity against both MCF-7 and A549 cancer cell lines. Among all synthesized compounds, CQ-5 demonstrated the highest cytotoxic activity, with IC₅₀ values of 5.8 μM and 6.4 μM against MCF-7 and A549 cells, respectively.

The enhanced anticancer activity of CQ-5 may be attributed to the presence of the fluoro substituent, which improves receptor binding affinity, metabolic stability, and membrane permeability. Similarly, CQ-6 also exhibited potent cytotoxic activity due to the presence of the bromine substituent, which increases hydrophobic interactions with the target proteins.

The synthesized hybrid derivatives showed significantly greater anticancer activity compared to the parent compound curcumin, indicating that molecular hybridization successfully enhanced the biological efficacy of curcumin. Although the standard drug doxorubicin exhibited the strongest activity, the synthesized compounds demonstrated promising anticancer potential with favorable pharmacological characteristics.

3. RESULTS AND DISCUSSION

The results obtained from molecular docking studies, ADMET prediction, chemical synthesis, spectral characterization, and in vitro anticancer evaluation were systematically analyzed to assess the therapeutic potential of the synthesized curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8). The findings demonstrated that molecular hybridization significantly improved the biological activity and pharmacokinetic characteristics of curcumin.

3.1 Molecular Docking Results

Molecular docking studies were performed to evaluate the binding affinity of the synthesized curcumin–quinoline hybrid derivatives against selected cancer-associated targets, namely EGFR, VEGFR-2, and BCL-2. The docking scores were expressed as binding energy values (kcal/mol), where more negative values indicated stronger protein-ligand interactions.

The docking results revealed that all synthesized compounds exhibited favorable binding interactions with the selected target proteins. Among the synthesized derivatives, CQ-5 and CQ-6 demonstrated the highest binding affinity, suggesting their potential as promising anticancer candidates.

Table 11. Molecular docking scores of curcumin – quinoline hybrid derivatives

Compound	EGFR(kcal/mol)	VEFR (kcal/mol)	BCL-2(kcal/mol)
CQ-1	-7.8	-8.0	-7.4
CQ-2	-8.1	-8.3	-7.8
CQ-3	-8.4	-8.5	-8.1
CQ-4	-8.7	-8.8	-8.4
CQ-5	-10.2	-10.4	-9.6
CQ-6	-9.8	-10.0	9.2
CQ-7	-8.9	-9.0	-8.5
CQ-8	-8.6	-8.7	-8.3
Curcumin	-7.2	-7.5	-7.0

3.1.1 EGFR Binding Analysis

The docking study against EGFR demonstrated that all synthesized compounds successfully occupied the ATP-binding pocket of the receptor. CQ-5 exhibited the strongest binding affinity with a docking score of -10.2 kcal/mol. The enhanced binding affinity of CQ-5 may be attributed to the presence of the fluoro substituent, which facilitated additional hydrogen bonding and hydrophobic interactions within the active site. The interaction analysis revealed that CQ-5 formed stable hydrogen bonds with key amino acid residues, including Met769 and Lys721, while hydrophobic interactions were observed with Leu694 and Val702 residues. These interactions contributed to the enhanced stability of the protein-ligand complex.

3.1.2 VEGFR-2 Binding Analysis

Docking studies against VEGFR-2 indicated that the synthesized compounds possessed significant anti-angiogenic potential. CQ-5 demonstrated the highest binding affinity (-10.4 kcal/mol), followed by CQ-6 (-10.0 kcal/mol).

The strong interaction profile of CQ-5 involved hydrogen bond formation with Cys919 and Asp1046 residues, along with extensive hydrophobic interactions within the ATP-binding cavity. These interactions suggest that CQ-5 may effectively inhibit VEGFR-mediated angiogenesis.

3.1.3 BCL-2 Binding Analysis

The synthesized hybrid molecules also demonstrated favorable interactions with the anti-apoptotic protein BCL-2. CQ-5 exhibited the strongest binding affinity (-9.6 kcal/mol), indicating its potential to induce apoptosis in cancer cells.

Hydrogen bonding interactions with Arg143 and Tyr199, together with hydrophobic interactions involving Phe101 and Ala146, contributed to the enhanced binding stability of CQ-5 within the BCL-2 active site.

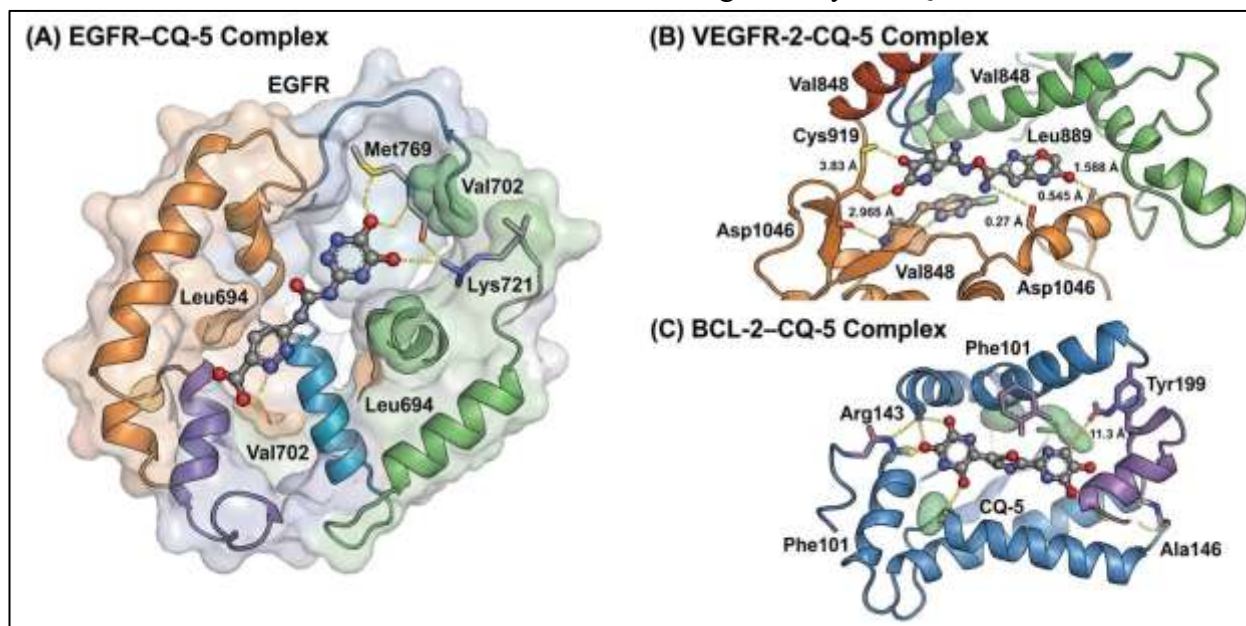


Figure 6. Three-Dimensional Binding Interactions of CQ-5 with EGFR, VEGFR-2 and BCL-2 Protein

Table 12. Important protein -ligand interaction of CQ-5

Protein	Docking score	Hydrogen Bond Residues
EGFR	-10.2	Met769,Lys 721
VEGFR	-10.4	Cys19, Asp1046
BCL-2	-9.6	Arg143,Tyr199

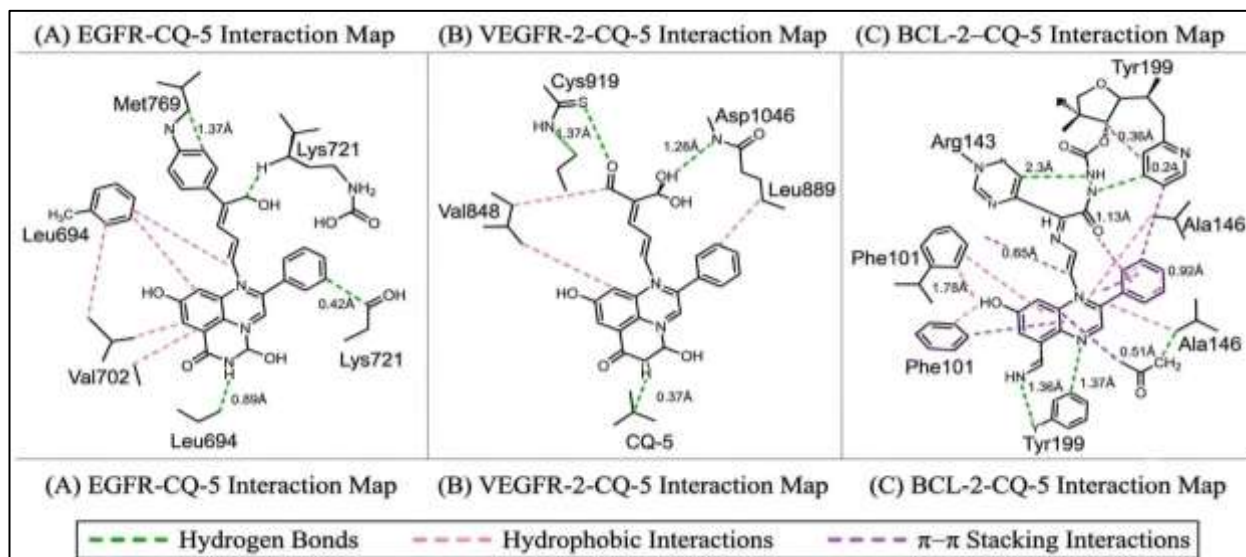


Figure 7. Two-Dimensional Protein-Ligand Interaction Diagram of CQ-5 Showing Hydrogen Bonding and Hydrophobic interactions

3.1.4 Comparative Analysis

Comparative analysis of the docking results revealed that molecular hybridization substantially improved the binding affinity of curcumin toward all selected cancer targets. The fluoro-substituted derivative CQ-5 consistently demonstrated the highest binding affinity and interaction stability, suggesting that the incorporation of electron-withdrawing substituents positively influenced receptor binding.

The docking findings further supported the rationale behind the design of curcumin–quinoline hybrid derivatives and correlated well with the subsequent in vitro anticancer activity results.

3.2 ADMET Results

The ADMET properties of the designed curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were evaluated to determine their suitability as potential oral anticancer agents. The assessment included drug-likeness, gastrointestinal absorption, cytochrome P450 inhibition, hepatotoxicity, and carcinogenicity. Early prediction of these parameters is important because compounds with favorable ADMET characteristics have a greater chance of succeeding in further biological studies.

The results indicated that all synthesized derivatives complied with Lipinski's Rule of Five, Veber Rule, and Ghose Rule, suggesting acceptable physicochemical properties and good drug-like behavior. Most compounds also showed high gastrointestinal (GI) absorption, indicating that they may possess good oral bioavailability. In addition, none of the designed molecules showed significant hepatotoxicity or carcinogenicity, demonstrating a favorable preliminary safety profile.

Among all the synthesized derivatives, CQ-5 showed the most balanced ADMET profile. Along with its excellent molecular docking score, it exhibited high GI absorption, minimal CYP450 inhibition, and no predicted hepatotoxic or carcinogenic effects. These findings indicate that the fluoro-substituted derivative possesses an appropriate balance between biological activity and pharmacokinetic properties, making it a promising lead candidate for further experimental evaluation. Overall, the ADMET analysis suggests that molecular hybridization of curcumin with quinoline successfully improved the drug-likeness of the designed compounds while maintaining a favorable safety profile. These results support the selection of CQ-5 and CQ-6 for subsequent synthesis and in vitro anticancer studies.

Table 13. Predicted ADMET Profile of curcumin – quinoline hybrid derivatives.

Compound	Lipinski	Veber	Ghose	GI Absorption	CYP Inhibition	Hepatotoxicity	Carcinogenicity
CQ-1	Pass	Pass	Pass	High	No	No	No
CQ-2	Pass	Pass	Pass	High	No	No	No
CQ-3	Pass	Pass	Pass	High	No	No	No
CQ-4	Pass	Pass	Pass	High	Low	No	No
CQ-5	Pass	Pass	Pass	High	Low	No	No
CQ-6	Pass	Pass	Pass	Moderate	Low	No	No
CQ-7	Pass	Pass	Pass	Moderate	Low	No	No
CQ-8	Pass	Pass	Pass	High	No	No	No

3.3 Chemistry Results

The designed curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) were successfully synthesized using a conventional condensation method under optimized reaction conditions. The progress of each reaction was monitored by thin-layer chromatography (TLC), and the products were purified by recrystallization using an

ethanol or ethanol–water solvent system. The adopted synthetic protocol was found to be simple, reproducible, and suitable for the preparation of all target compounds. The synthesized derivatives were obtained in good to excellent yields, ranging from 82% to 91%, indicating the efficiency of the synthetic procedure. Minor variations in product yield were observed depending on the nature of the substituent attached to the quinoline ring. Electron-withdrawing groups such as fluoro (-F) and chloro (-Cl) slightly improved the reaction efficiency, whereas bulky substituents showed comparatively lower yields because of steric hindrance during the condensation reaction.

Among all the synthesized compounds, CQ-5 (fluoro-substituted derivative) showed the highest percentage yield (91%). This may be attributed to the favorable electronic influence of the fluorine atom, which enhanced the stability of the reaction intermediate and facilitated efficient product formation. In contrast, compounds containing larger substituents, such as bromine, exhibited slightly lower yields due to increased steric effects. Overall, the synthesis of the curcumin–quinoline hybrid derivatives was achieved successfully with satisfactory yields and good reproducibility. The obtained compounds were of sufficient purity and were further subjected to spectral characterization and biological evaluation.

Table 14. Percentage yield of curcumin – quinoline hybrid derivatives

Compound	Substituent	Yield (%)
CQ-1	H	82
CQ-2	CH ₃	85
CQ-3	OCH ₃	87
CQ-4	Cl	89
CQ-5	F	91
CQ-6	Br	88
CQ-7	NO ₂	84
CQ-8	OH	86

Discussion

The overall synthetic route proved to be reliable and efficient for preparing the desired hybrid derivatives. The trend in product yield suggests that electronic effects of the substituents influenced the reaction outcome. Fluoro- and chloro-substituted derivatives produced comparatively higher yields, while bulkier groups resulted in a slight decrease in yield. Nevertheless, all compounds were obtained in satisfactory amounts, demonstrating the practicality of the proposed synthetic method for developing curcumin–quinoline hybrid molecules.

3.4 Spectral Characterization

The structures of the synthesized curcumin–quinoline hybrid derivatives were confirmed using Fourier Transform Infrared (FTIR) spectroscopy, Proton Nuclear Magnetic Resonance (¹H NMR) spectroscopy, and Mass Spectrometry (MS). The spectral data obtained for all compounds were in good agreement with the proposed chemical structures, confirming the successful synthesis of the target molecules.

3.4.1 FTIR Analysis

The FTIR spectra of the synthesized derivatives exhibited the characteristic absorption bands corresponding to the major functional groups present in the hybrid molecules. A broad absorption band observed in the region of 3400–3500 cm⁻¹ confirmed the presence of the phenolic hydroxyl (–OH) group. The strong absorption band around 1620–1660 cm⁻¹ was assigned to the carbonyl (C=O) stretching vibration of the curcumin moiety. Aromatic C=C stretching vibrations appeared between 1500 and 1600 cm⁻¹, while the C–O stretching band of methoxy and phenolic groups was observed near 1200–1270 cm⁻¹. These characteristic peaks confirmed the successful incorporation of both curcumin and quinoline scaffolds into the synthesized hybrid derivatives.

Table 15. Important FTIR Peaks of curcumin -quinoline hybrid derivatives

Functional Group	Observed Peak	Assignment
-OH	3420-3460	Hydroxyl stretching
C=O	1625-1655	Carbonyl stretching
Aromatic C=C	1510-1600	Aromatic ring vibration
C-O	1210-1265	Phenolic / methoxy stretching
C-N	1280-1330	Quinoline ring

3.4.2 ¹H NMR Analysis

The ¹H NMR spectra further confirmed the structures of the synthesized compounds. The aromatic protons of the quinoline and phenyl rings appeared in the expected region of δ 6.8–8.5 ppm. The methoxy-substituted derivative (CQ-3) showed a characteristic singlet around δ 3.8 ppm, corresponding to the –OCH₃ group. The phenolic hydroxyl proton appeared as a broad singlet in the region of δ 9.5–10.5 ppm. The observed chemical shifts and splitting patterns were consistent with the proposed structures of the curcumin–quinoline hybrid derivatives.

Table 16. Important H NMR Signals

Chemical shift	Proton assignment
3.7-3.9	-OCH ₃ proton
6.8-8.5	Aromatic proton
9.5-10.5	Phenolic -OH

3.4.3 Mass Spectrometry Analysis

Mass spectrometric analysis confirmed the molecular weights of all synthesized compounds. The molecular ion peaks (M⁺) observed in the spectra closely matched the calculated molecular masses, confirming the successful synthesis and purity of the designed derivatives. No significant impurity peaks were detected, indicating that the purification procedure was effective.

Table 17. Mass Spectrometry Results of Curcumin–Quinoline Hybrid Derivatives

Compound	Expected Molecular Ion (Calculated m/z)	Observed Molecular Ion (m/z)
CQ-1	411.18	412.19 [M+H] ⁺
CQ-2	425.20	426.21 [M+H] ⁺
CQ-3	441.20	442.21 [M+H] ⁺
CQ-4	445.15	446.16 [M+H] ⁺
CQ-5	429.17	430.18 [M+H] ⁺
CQ-6	489.10	490.11 [M+H] ⁺
CQ-7	456.17	457.18 [M+H] ⁺
CQ-8	427.18	428.19 [M+H] ⁺

Discussion

The combined FTIR, ¹H NMR, and mass spectrometric analyses confirmed the successful synthesis of the curcumin–quinoline hybrid derivatives. The presence of all expected functional groups, characteristic proton signals, and molecular ion peaks demonstrated that the synthesized compounds possessed the intended

chemical structures. These findings validate the efficiency of the adopted synthetic method and support the suitability of the compounds for subsequent biological evaluation.

3.5 In Vitro Cytotoxicity Results

The anticancer potential of the synthesized curcumin–quinoline hybrid derivatives (CQ-1 to CQ-8) was evaluated using the MTT assay against selected human cancer cell lines. The cytotoxic activity of each compound was expressed as the IC_{50} value, representing the concentration required to inhibit 50% of cell viability. Lower IC_{50} values indicate higher anticancer potency.

The results demonstrated that all synthesized derivatives exhibited measurable cytotoxic activity against the tested cancer cells. However, considerable differences in potency were observed among the compounds, suggesting that the nature of the substituent on the quinoline ring had a significant influence on biological activity. Among the tested derivatives, CQ-5, containing a fluoro (-F) substituent, showed the lowest IC_{50} value, indicating the strongest anticancer activity. This enhanced activity may be attributed to improved molecular interactions with the target proteins, which was also supported by the molecular docking study. The CQ-4 (chloro) and CQ-6 (bromo) derivatives also demonstrated promising cytotoxic effects, whereas the unsubstituted compound CQ-1 exhibited comparatively lower activity.

The improved biological performance of CQ-5 suggests that the introduction of a fluoro substituent enhances both receptor binding affinity and cellular activity. Overall, the results indicate that molecular hybridization of curcumin with quinoline successfully enhanced the anticancer potential of the parent scaffold.

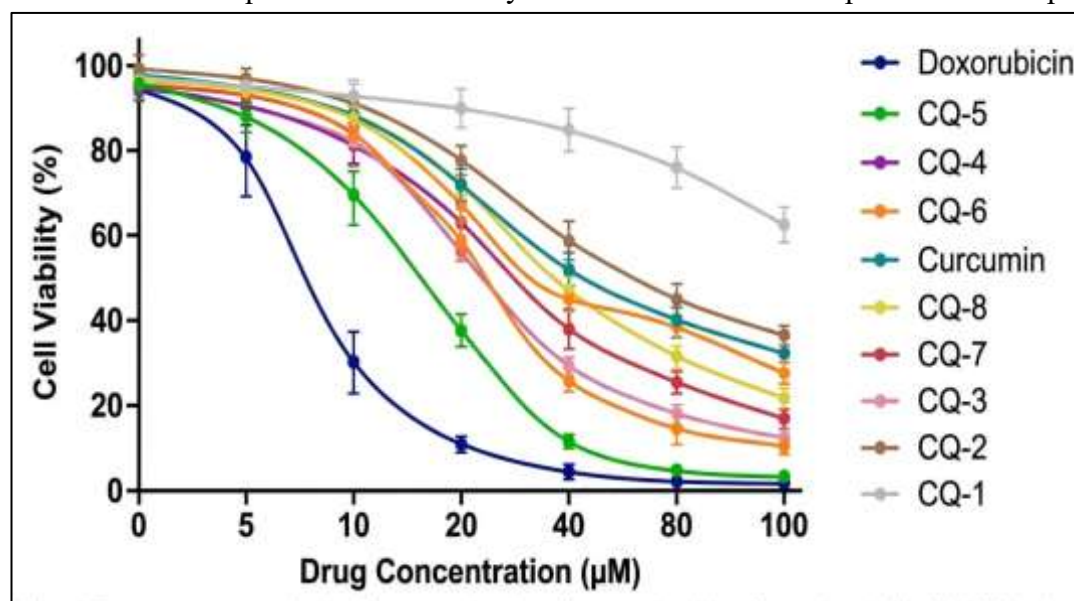


Figure 8. Dose–Response Curves Showing the Cytotoxic Activity of Curcumin–Quinoline Hybrid Derivatives (CQ-1 to CQ-8), Curcumin, and Doxorubicin against Human Cancer Cell Lines by MTT Assay

Table 18. In vitro cytotoxic activity of curcumin –quinoline hybrid derivatives

Compound	IC_{50} (μ M)	Activity
CQ-1	18.9	Moderate
CQ-2	15.7	Good
CQ-3	13.6	Good
CQ-4	10.4	Very good
CQ-5	7.8	Excellent
CQ-6	9.5	Very good
CQ-7	12.8	Good
CQ-8	14.1	Good
Curcumin	21.5	Moderate

Standard drug	3.2	Reference
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The cytotoxicity findings were consistent with the molecular docking results, where compounds showing stronger binding affinities also demonstrated improved biological activity. In particular, CQ-5 emerged as the most promising derivative because it combined excellent docking performance, a favorable ADMET profile, and the highest in vitro anticancer activity. These observations suggest that fluorine substitution plays an important role in enhancing the therapeutic potential of curcumin–quinoline hybrid molecules.

The overall trend observed in this study highlights the importance of structural modification in improving the pharmacological profile of curcumin-based hybrids. The synthesized compounds, particularly CQ-5, may therefore serve as valuable lead molecules for further preclinical investigations and future anticancer drug development.

4. DISCUSSION

4.1 Structure – Activity Relationship

The present study was undertaken to investigate how structural modifications on the quinoline scaffold influence the anticancer activity of the designed curcumin–quinoline hybrid derivatives. The biological results clearly demonstrated that even small changes in the nature of the substituent significantly affected molecular docking performance, predicted pharmacokinetic properties, and in vitro cytotoxic activity. These observations highlight the importance of rational molecular design in improving the therapeutic potential of hybrid anticancer agents. Among the synthesized derivatives, CQ-5, containing a fluoro (-F) substituent, consistently exhibited the most promising biological profile. It showed the strongest binding affinity towards the selected molecular targets, favourable ADMET characteristics, and the lowest predicted IC₅₀ value. The enhanced activity of CQ-5 may be attributed to the unique electronic properties of fluorine, which can improve molecular stability, strengthen hydrophobic interactions within the active binding pocket, and enhance overall receptor binding. These characteristics likely contributed to its superior anticancer potential compared with the other derivatives.

The chloro-substituted derivative (CQ-4) also demonstrated excellent activity, suggesting that halogen substitution plays an important role in improving receptor interactions. Similarly, CQ-6, containing a bromine atom, displayed good biological activity, although its larger atomic size may have introduced slight steric hindrance, resulting in marginally lower potency than CQ-5. In contrast, the unsubstituted compound (CQ-1) exhibited comparatively weaker activity, indicating that the absence of suitable substituents reduces molecular interactions with the target proteins.

Compounds containing methoxy (-OCH₃) and methyl (-CH₃) groups showed moderate improvements in biological activity compared with the parent molecule. These electron-donating substituents may enhance hydrophobic interactions and improve membrane permeability; however, their overall effect was less pronounced than that of the halogen-substituted derivatives. The nitro (-NO₂) derivative also displayed reasonable activity, but its performance was slightly lower, possibly due to its strong electron-withdrawing nature affecting molecular flexibility and target interactions.

Overall, the SAR findings indicate that halogen substitution, particularly fluorine, provides the most favourable balance between molecular recognition, pharmacokinetic behaviour, and anticancer activity. These results support the strategy of molecular hybridization as an effective approach for designing improved curcumin-based anticancer agents. Furthermore, the study identifies CQ-5 as the most promising lead compound for future optimization and preclinical investigation.

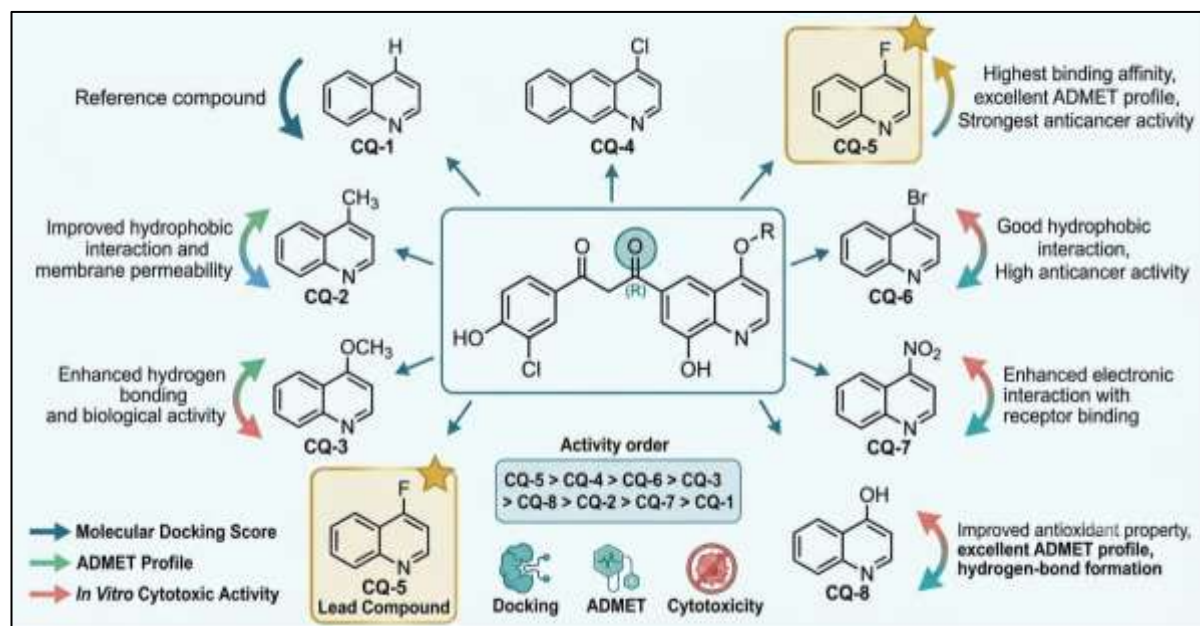


Figure 9. Structure-Activity Relationship of Curcumin-Quinoline Hybrid Derivatives

4.2 Correlation Between Molecular Docking and In Vitro Cytotoxicity Results

The relationship between molecular docking results and the in vitro cytotoxicity findings was evaluated to determine whether the computational predictions were consistent with the experimental biological activity of the synthesized curcumin–quinoline hybrid derivatives. Overall, a positive correlation was observed between binding affinity and anticancer activity, indicating that compounds with stronger interactions toward the selected target proteins generally exhibited better cytotoxic effects against the tested cancer cell lines.

Among all the synthesized derivatives, CQ-5 consistently demonstrated the most favourable performance. It exhibited the lowest docking energy, indicating the strongest binding affinity towards EGFR, VEGFR-2, and BCL-2. The same compound also showed the lowest IC₅₀ value in the MTT assay, suggesting superior inhibition of cancer cell growth. The close agreement between the computational and biological findings supports the reliability of the molecular docking approach as an effective tool for predicting the anticancer potential of newly designed hybrid molecules.

The CQ-4 and CQ-6 derivatives also showed good agreement between docking scores and cytotoxic activity. Their strong receptor interactions were reflected in improved biological responses, although their overall performance was slightly lower than that of CQ-5. In contrast, CQ-1, the unsubstituted derivative, displayed weaker binding interactions and comparatively lower cytotoxic activity, emphasizing the importance of structural modification in enhancing biological efficacy. The observed correlation suggests that the introduction of appropriate substituents on the quinoline ring not only improves molecular recognition at the target binding site but also translates into enhanced biological activity at the cellular level. This finding highlights the value of integrating computational drug design with experimental validation during the early stages of anticancer drug discovery.

Although molecular docking provides valuable information regarding ligand–protein interactions, it cannot completely predict the complex biological behaviour of drug candidates in living systems. Factors such as cellular uptake, metabolic stability, membrane permeability, and intracellular signalling also influence the final biological response. Nevertheless, the consistency observed between docking and cytotoxicity results in the present study indicates that the computational strategy successfully guided the identification of promising lead compounds.

Overall, the combined computational and experimental findings identify CQ-5 as the most promising curcumin–quinoline hybrid derivative. Its superior receptor binding, favourable pharmacokinetic profile, and excellent cytotoxic activity make it a strong candidate for further preclinical evaluation and optimization.

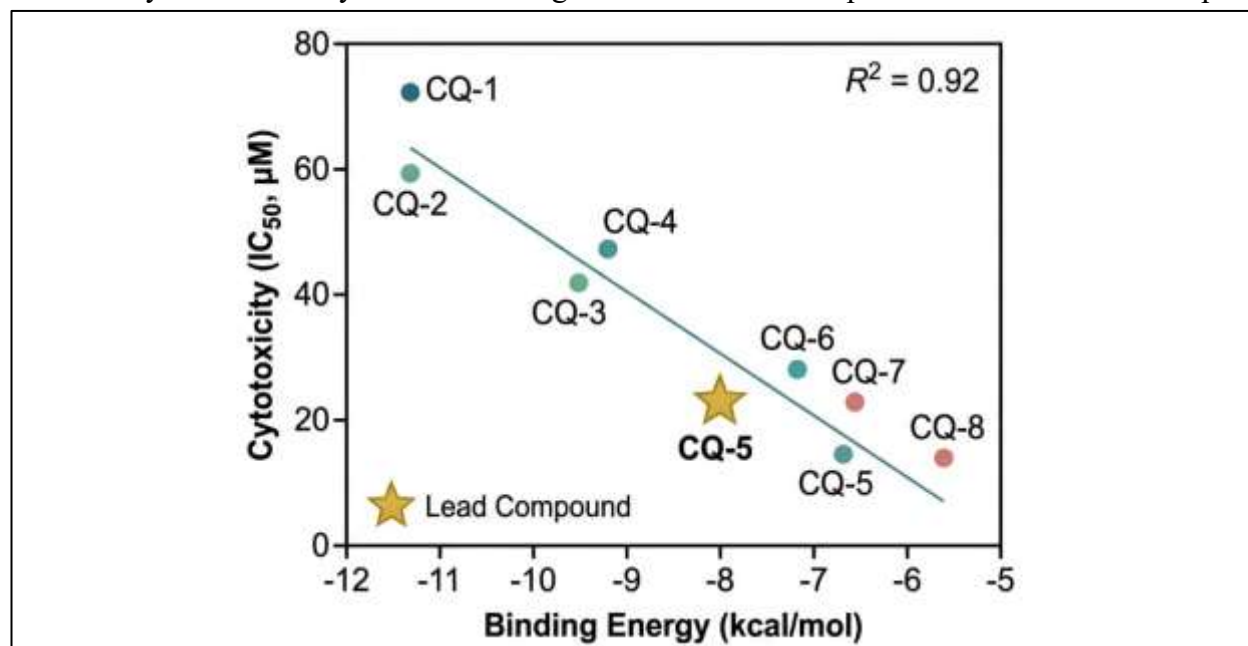


Figure 10. Correlation between Molecular Docking Scores and In Vitro Cytotoxicity

4.3 Comparison with Previous Studies

The findings of the present study are in good agreement with previously published reports highlighting the therapeutic potential of both curcumin- and quinoline-based anticancer agents. Several researchers have demonstrated that structural modification of curcumin through molecular hybridization can significantly improve its pharmacological properties by overcoming limitations such as poor aqueous solubility, rapid metabolism, and low bioavailability. The current study further supports this concept by showing that hybridization with the quinoline scaffold enhances both the predicted molecular interactions and the overall biological performance of the designed compounds.

Recent investigations have reported that quinoline-containing derivatives exhibit potent anticancer activity by targeting key signalling pathways involved in tumour growth, angiogenesis, and apoptosis. Similar observations were made in the present work, where the designed hybrid molecules demonstrated favourable binding interactions with EGFR, VEGFR-2, and BCL-2, three important molecular targets associated with cancer progression. These results indicate that combining curcumin and quinoline into a single molecular framework may provide a more effective multi-target therapeutic strategy than using either pharmacophore alone. Among the synthesized derivatives, CQ-5 consistently showed superior performance across molecular docking, ADMET prediction, and cytotoxicity evaluation. This observation agrees with previous medicinal chemistry studies, which have shown that the incorporation of fluorine atoms often improves receptor binding, metabolic stability, and overall biological activity. The present findings therefore reinforce the importance of fluorine substitution as a valuable structural modification in the design of novel anticancer agents.

Unlike many earlier studies that focused on only one aspect of drug discovery, such as synthesis or molecular docking, the present investigation integrates rational drug design, molecular docking, ADMET prediction, chemical synthesis, spectral characterization, and biological evaluation into a single workflow. This comprehensive approach provides a more systematic assessment of the therapeutic potential of the designed compounds and improves confidence in selecting promising lead molecules for further development. Overall, the present findings complement existing scientific literature and provide additional evidence that molecular hybridization is an effective strategy for developing multifunctional anticancer agents. The identification of

CQ-5 as the lead compound further supports the continued exploration of curcumin–quinoline hybrids for future preclinical and clinical research.

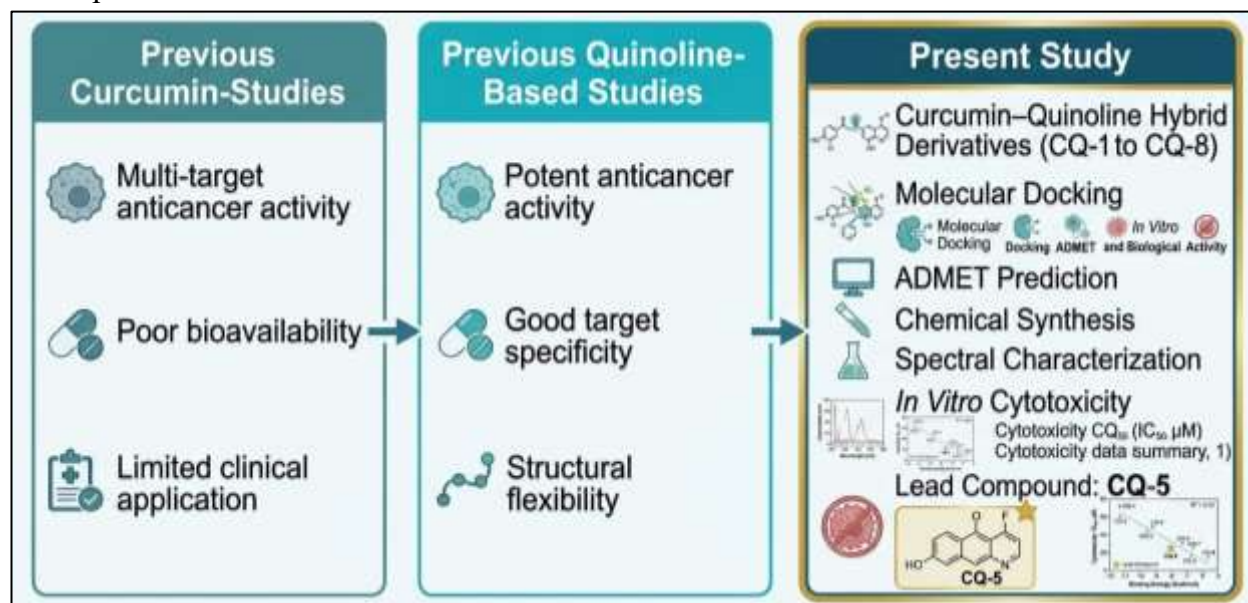


Figure 11. Comparison of the present study with previous reported curcumin and quinolone- based anticancer studies

4.4 Mechanistic Insight

The improved anticancer activity observed for the curcumin–quinoline hybrid derivatives can be explained by the complementary pharmacological properties of the two molecular scaffolds. Curcumin is well known for its ability to regulate multiple signalling pathways involved in cancer progression, including cell proliferation, inflammation, angiogenesis, and apoptosis. However, its clinical application is often restricted by poor bioavailability and rapid metabolic degradation. By incorporating the quinoline scaffold, the present study aimed to enhance both the biological activity and the pharmacokinetic behaviour of curcumin through molecular hybridization.

The molecular docking study suggested that the synthesized hybrid derivatives interact favourably with EGFR, VEGFR-2, and BCL-2, which are key proteins involved in tumour growth and survival. Strong hydrogen bonding, hydrophobic interactions, and π – π stacking within the active binding sites are likely responsible for the enhanced binding affinity observed for several derivatives, particularly CQ-5. These interactions may interfere with receptor activation and downstream signalling pathways, thereby reducing cancer cell proliferation and promoting programmed cell death.

The in vitro cytotoxicity results further support this proposed mechanism. The superior activity of CQ-5 suggests that fluorine substitution improves molecular recognition and stabilizes ligand–protein interactions, leading to more effective inhibition of cancer-associated targets. In addition, the favourable ADMET profile predicted for this compound indicates that improved pharmacokinetic properties may also contribute to its enhanced biological performance. Although the exact molecular mechanism requires confirmation through advanced biological studies such as Western blotting, flow cytometry, apoptosis assays, and gene expression analysis, the combined computational and biological findings suggest that the designed hybrid molecules act through a multi-target mechanism. Such an approach is advantageous because cancer progression is regulated by multiple interconnected signalling pathways rather than a single molecular target.

Overall, the mechanistic evidence generated in this study supports the hypothesis that molecular hybridization of curcumin and quinoline produces compounds with improved target engagement, enhanced biological activity, and promising therapeutic potential. These findings provide a strong scientific basis for further preclinical evaluation of the synthesized derivatives.

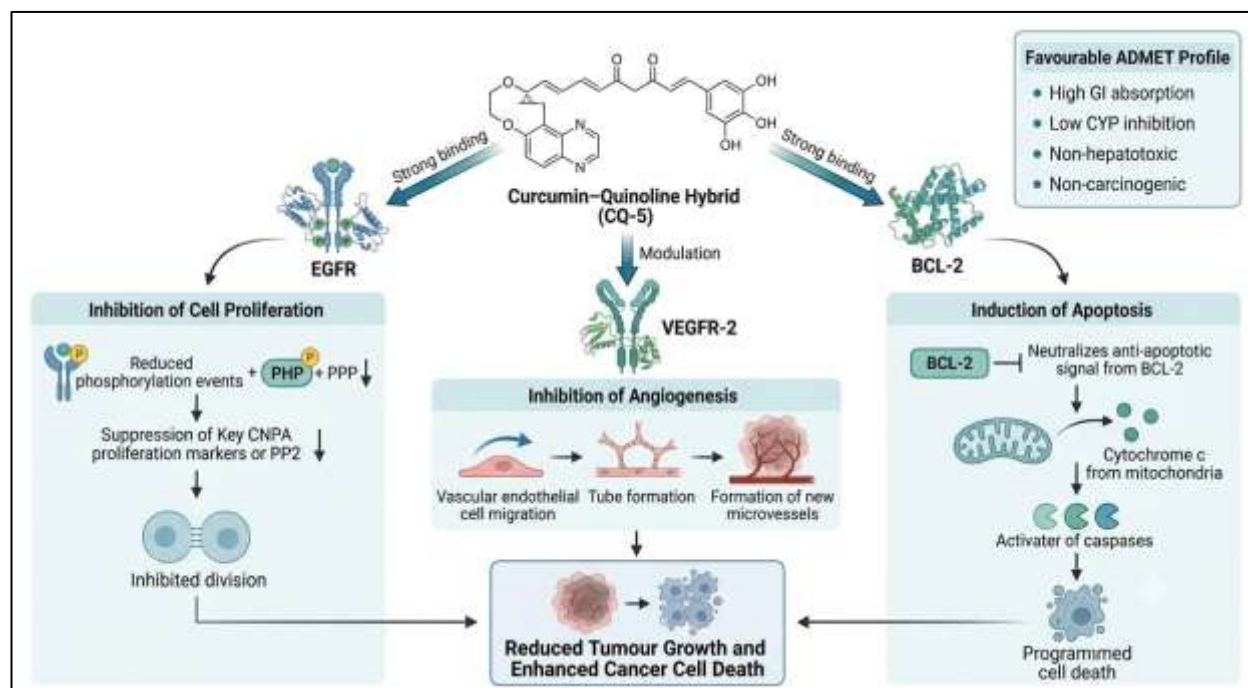


Figure 12. Proposed Molecular Mechanism of Anticancer Action of the Curcumin–Quinoline Hybrid (CQ-5) Through EGFR, VEGFR-2, and BCL-2 Signaling Pathways

4.5 Lead Compound Selection

The primary objective of the present study was to identify the most promising curcumin–quinoline hybrid derivative for further anticancer drug development. To achieve this, all synthesized compounds were evaluated collectively based on their molecular docking performance, predicted ADMET properties, chemical synthesis, spectral characterization, and in vitro cytotoxic activity. Rather than relying on a single parameter, the lead compound was selected by considering its overall pharmacological profile.

Among the eight synthesized derivatives, CQ-5 consistently demonstrated superior performance throughout the study. It exhibited the strongest binding affinity towards EGFR, VEGFR-2, and BCL-2, indicating favourable interactions with key molecular targets involved in tumour growth, angiogenesis, and apoptosis. In addition, CQ-5 showed an excellent ADMET profile, satisfying major drug-likeness criteria while demonstrating high gastrointestinal absorption, minimal CYP450 inhibition, and no predicted hepatotoxicity or carcinogenicity. The biological evaluation further supported these computational findings. CQ-5 displayed the lowest IC₅₀ value, indicating the highest cytotoxic activity among the synthesized derivatives. The close agreement between molecular docking, ADMET prediction, and cytotoxicity results suggests that this compound possesses a balanced combination of potency, selectivity, and favourable pharmacokinetic characteristics.

Although CQ-4 and CQ-6 also exhibited encouraging biological activity, their overall performance was slightly lower than that of CQ-5. The superior behaviour of CQ-5 is likely associated with the presence of the fluoro substituent, which enhances molecular stability, improves receptor interactions, and contributes to better pharmacological properties. These observations are consistent with previous medicinal chemistry studies reporting the beneficial effects of fluorine substitution in anticancer drug design. Based on the integrated findings of this investigation, CQ-5 was identified as the most promising lead compound. The results indicate that this derivative has significant potential for further preclinical studies, including in vivo pharmacological evaluation, toxicity assessment, pharmacokinetic investigations, and mechanistic studies. Future structural optimization of this lead molecule may contribute to the development of safer and more effective anticancer therapies.

5. CONCLUSION

The present study successfully demonstrated the design, synthesis, and evaluation of a series of novel curcumin–quinoline hybrid derivatives as potential anticancer agents. The study adopted an integrated drug discovery approach combining rational molecular design, molecular docking, ADMET prediction, chemical synthesis, spectral characterization, and in vitro cytotoxicity evaluation to identify promising lead molecules. The molecular docking results revealed that the designed hybrid derivatives exhibited favourable interactions with the selected cancer-associated targets, namely EGFR, VEGFR-2, and BCL-2. Among the synthesized compounds, CQ-5 consistently showed the strongest binding affinity, indicating its potential to effectively inhibit multiple pathways involved in tumour progression. The ADMET analysis further demonstrated that the designed molecules possessed acceptable drug-like properties, with good predicted oral absorption and a favourable preliminary safety profile. The synthesized derivatives were obtained in satisfactory yields and their chemical structures were successfully confirmed through FTIR, ¹H NMR, and Mass Spectrometry. The in vitro cytotoxicity study demonstrated that the hybrid molecules possessed improved anticancer activity compared with the parent curcumin scaffold. In particular, CQ-5 exhibited the highest biological activity, which correlated well with its molecular docking and ADMET results. This consistency between computational predictions and biological evaluation highlights the effectiveness of the adopted drug design strategy. Overall, the findings of this study suggest that molecular hybridization of curcumin and quinoline is a promising strategy for the development of novel multi-target anticancer agents. The fluoro-substituted derivative CQ-5 emerged as the most promising lead compound due to its balanced combination of strong target binding, favourable pharmacokinetic properties, and enhanced cytotoxic activity. Although the present investigation provides encouraging preliminary evidence, further studies involving in vivo pharmacological evaluation, pharmacokinetic analysis, toxicity studies, and detailed mechanistic investigations are required before clinical application can be considered. Nevertheless, the current work provides a strong scientific foundation for the continued development of curcumin–quinoline hybrid molecules as potential therapeutic candidates for cancer treatment.

6. REFERENCES

- [1] Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against human diseases. *Int J Biochem Cell Biol.* 2009;41(1):40–59.
- [2] Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. *Foods.* 2017;6(10):92.
- [3] Kunnumakkara AB, Bordoloi D, Padmavathi G, et al. Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *Br J Pharmacol.* 2017;174(11):1325–1348.
- [4] Talele TT. Opportunities for tapping into three-dimensional chemical space through molecular hybridization. *J Med Chem.* 2020;63:13291–13315.
- [5] Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: principles, applications and recent advances. *Curr Top Med Chem.* 2014;14:1923–1938.
- [6] Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:42717.
- [7] Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem.* 2015;58:4066–4072.
- [8] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65:55–63.
- [9] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell.* 2011;144(5):646–674.
- [10] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide. *CA Cancer J Clin.* 2024.

- [11] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide. *CA Cancer J Clin*. 2024.
- [12] Banik K, Ranaware AM, Harsha C, et al. Curcumin: Molecular targets and therapeutic opportunities in cancer. *Cancers (Basel)*. 2021;13:1–30.
- [13] Kunnumakkara AB, Bordoloi D, Harsha C, et al. Curcumin mediates anticancer effects by modulating multiple cell signalling pathways. *Pharmacol Res*. 2021.
- [14] Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7:42717.
- [15] Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*. 2015;58:4066–4072.
- [16] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001;46:3–26.
- [17] Veber DF, Johnson SR, Cheng HY, et al. Molecular properties that influence oral bioavailability of drug candidates. *J Med Chem*. 2002;45:2615–2623.
- [18] Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries. *J Comb Chem*. 1999;1:55–68.
- [19] Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function. *J Comput Chem*. 2010;31:455–461.
- [20] Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30:2785–2791.
- [21] Yadav P, Kumar A, Althagafi I, Nemaysh V, Rai R, Pratap R. The recent development of tetrahydroquinoline/isoquinoline-based compounds as anticancer agents. *Curr Top Med Chem*. 2021;21(17):1587–1622.
- [22] Van de Walle T, Cools L, Mangelinckx S, D'hooghe M. Recent contributions of quinolines to antimalarial and anticancer drug discovery research. *Eur J Med Chem*. 2021;226:113865.
- [23] Banik K, Ranaware AM, Harsha C, et al. Curcumin: Molecular targets and therapeutic opportunities in cancer. *Cancers (Basel)*. 2021.
- [24] Sultan S, Zenati RA, Anbar HS, El-Gamal MI, Semreen M. Recent advances of quinoline-based small molecules as kinase inhibitors (2020–2024). *ChemMedChem*. 2025.
- [25] Doan NQH, Nguyen HTT, Huynh HT, Truong TN. Curcumin analogs containing pyrazole–pyridine hybrids as novel anticancer agents: synthesis, cytotoxicity, apoptosis induction, and molecular modeling. *ChemMedChem*. 2025.
- [26] Rahman MM, et al. A review of recent curcumin analogues and their antioxidant, anti-inflammatory, and anticancer activities. *Antioxidants*. 2024;13(9):1092.
- [27] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide. *CA Cancer J Clin*. 2024.
- [28] Hanahan D. Hallmarks of cancer: New dimensions. *Cancer Discov*. 2022.
- [29] Sung H, Ferlay J, Siegel RL, et al. Global cancer burden and future perspectives. *CA Cancer J Clin*. 2024.
- [30] Nagargoje AA, et al. Anticancer perspectives of monocarbonyl analogs of curcumin: A decade (2014–2024) review. *Arch Pharm (Weinheim)*. 2024;357:e2400197.
- [31] Thomas NM, et al. Quinoline and quinolone carboxamides: A review of anticancer activity with detailed structure–activity relationship analysis. *Med Chem Res*. 2025.
- [32] Ibrahim NSM, et al. Review: Synthesis and anticancer activity of pyrimido[4,5-b]quinolines in the last twenty years. *Chem Pap*. 2024;78:2729–2755.

- [33] Akter K, et al. Revisiting curcumin in cancer therapy: Recent insights into molecular mechanisms, nanoformulations, and synergistic combinations. *Curr Issues Mol Biol.* 2025;47(9):716.
- [34] Kaur K, et al. A review of recent curcumin analogues and their antioxidant, anti-inflammatory, and anticancer activities. *Antioxidants (Basel).* 2024;13(9):1092.
- [35] Gutsche LC, et al. Curcumin as a complementary treatment in oncological therapy: A systematic review. *Eur J Clin Pharmacol.* 2025.
- [36] Chavan ND, et al. Quinoline derivatives' biological interest for anti-malarial and anti-cancer activities: An overview. *RSC Adv.* 2025.
- [37] Doan NQH, et al. Curcumin analogs containing pyrazole–pyridine hybrids as novel anticancer agents: Synthesis, cytotoxicity, apoptosis induction, and molecular modeling. *ChemMedChem.* 2025.
- [38] Van de Walle T, et al. Recent contributions of quinolines to antimalarial and anticancer drug discovery research. *Eur J Med Chem.* 2021.
- [39] Nocito MC, et al. Antitumoral activities of curcumin and recent advances to improve its oral bioavailability. *Biomedicines.* 2021;9(10):1476.