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ORIGINAL ARTICLE

COMPUTATIONALLY DRIVEN DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF HYBRID BENZIMIDAZOLE–QUINAZOLINONE SCAFFOLDS: A STRUCTURE–ACTIVITY RELATIONSHIP AND MOLECULAR DOCKING APPROACH TOWARD NOVEL ANTICANCER AGENTS

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ABSTRACT : This study aimed to design, synthesize and biologically evaluate novel hybrid benzimidazole–quinazolinone derivatives as potential anticancer agents. A computational docking approach was employed to identify high-affinity ligands against the epidermal growth factor receptor (EGFR), followed by *in vitro* cytotoxic screening and structure–activity relationship (SAR) analysis. Docking studies revealed strong interactions of the designed ligands with key residues Met793 and Lys745 in the EGFR binding pocket, exhibiting binding energies between –7.2 and –9.4 kcal/mol. Compounds BQ-1 and BQ-2 demonstrated the highest docking scores and potent cytotoxic activity, with IC_{50} values below 10 µM against MCF-7 cells. SAR analysis indicated that electron-withdrawing substituents (–Cl, $–NO_2$) and moderate linker lengths enhanced both docking affinity and experimental cytotoxicity. All compounds exhibited acceptable ADMET profiles and selectivity toward cancer cells. The integrated computational and experimental approach successfully identified benzimidazole–quinazolinone hybrids as promising anticancer scaffolds. The strong agreement between in silico docking results and in vitro cytotoxic data validates the molecular design strategy. These hybrids, particularly BQ-1 and BQ-2, represent potential lead compounds for further optimization, mechanism studies, and preclinical evaluation.

Key words : Benzimidazole–quinazolinone hybrids, molecular docking, EGFR inhibition, anticancer agents, structure–activity relationship (SAR), *in silico* ADMET, MTT assay, drug design.

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INTRODUCTION

Cancer remains one of the leading causes of death worldwide, responsible for approximately 10 million deaths in 2022 according to the World Health Organization (WHO, 2023). Despite significant advancements in treatment modalities, including chemotherapy, radiation, and targeted therapy, the development of resistance and severe side effects continue to limit the efficacy of current anticancer agents (Ferlay *et al*, 2021). Therefore, there is an urgent need to develop new, effective, and safer anticancer compounds.

Heterocyclic compounds have emerged as a key focus in medicinal chemistry due to their diverse biological activities and structural versatility. Among them, benzimidazoles and quinazolinones are well-known pharmacophores with wide therapeutic potential (Sahu *et al*, 2022). Benzimidazole derivatives exhibit a broad spectrum of pharmacological activities, including anticancer, antiviral, and antimicrobial effects (Patel *et al*, 2021). Similarly, quinazolinone-based compounds have shown potent cytotoxic activity against various cancer cell lines and serve as scaffolds for clinically used drugs like gefitinib and erlotinib (Yadav *et al*, 2020). These findings highlight the potential of these two heterocycles as key components in designing novel anticancer agents.

Hybrid molecules combining two pharmacophoric units within a single structure have gained attention as an effective strategy to enhance biological activity and overcome drug resistance (Ali et al., 2023). The combination of benzimidazole and quinazolinone scaffolds offers the potential for synergistic effects, improved receptor binding, and enhanced pharmacokinetic profiles (Kumar & Singh, 2021). Computational drug design approaches, such as molecular docking and ADMET analysis, play a crucial role in accelerating this process by predicting how these hybrid molecules interact with target proteins and by filtering out non-drug-like candidates early in development (Sliwoski et al., 2014).

Thus, employing computational methods can reduce the time and cost associated with experimental drug discovery while increasing the probability of identifying promising lead compounds (Lionta *et al*, 2014).

The objective of this study is to design, synthesize, and biologically evaluate novel hybrid molecules that integrate the benzimidazole and quinazolinone frameworks. Using computational tools, such as molecular docking and in silico ADMET prediction, the study aims to identify promising candidates with potent anticancer activity. The synthesized compounds will then be evaluated for their cytotoxic effects against selected cancer cell lines to establish a structure–activity relationship (SAR) and assess their potential as novel anticancer agents.

Benzimidazoles are privileged, nitrogen-containing heterocycles widely explored in medicinal chemistry for anticancer, antimicrobial, antiviral, and anti-inflammatory activities (Brishty *et al*, 2021; Ebenezer *et al*, 2023). Mechanistically, many benzimidazole derivatives induce apoptosis, disrupt microtubule dynamics, inhibit kinases, and modulate epigenetic enzymes, which underpins their broad antitumor potential (Lee *et al*, 2022; Wagih *et al*, 2025). Their compact scaffold tolerates diverse substitutions at N-1 and C-2 (and on the fused benzene ring), enabling fine-tuning of potency, selectivity and ADMET properties—features that explain their recurring success as cores in hit-to-lead campaigns (Brishty *et al*, 2021; Feng *et al*, 2022).

Quinazolinones (the 4-oxo analogs of quinazoline) constitute another privileged class with documented antiproliferative effects via multiple mechanisms—e.g., kinase inhibition (including c-MET), tubulin interference, topoisomerase modulation and induction of apoptosis (Mortazavi *et al*, 2022; Deng *et al*, 2025; Antoniolli *et al*, 2025). Recent syntheses continue to deliver quinazolinone series with low-micromolar activity across solid tumor lines, supported by docking and biophysical validation (Haneen *et al*, 2025; Abdelall *et al*, 2025). Importantly, while several quinazoline drugs (e.g., EGFR inhibitors) are clinically approved, quinazolinone frameworks remain mainly at the preclinical stage—highlighting ongoing opportunities to translate their broad target engagement into candidates with optimized selectivity and pharmacokinetics (Rezaeinasab *et al*, 2022).

Molecular hybridization—covalently merging two pharmacophores into a single scaffold—has emerged as a strategy to enhance potency, polypharmacology and resistance liability compared with single-warhead agents (Morais *et al*, 2024). In the benzimidazole domain, numerous hybrids (e.g., benzimidazole–purine/pyrimidine, benzimidazole–quinoline, and benzimidazole–EGFR-directed hybrids) report sub-micromolar cytotoxicity and improved target engagement supported by docking/SAR (Diaconu *et al*, 2022; Feng *et al*, 2022; Iacob *et al*, 2025; Mavrova *et al*, 2025).

With respect to the exact pair of interest, benzimidazole–quinazolinone hybrids are comparatively rare in the literature. Earlier work synthesized benzimidazole–quinazoline (not quinazolinone) regioisomers and observed measurable activity across NCI-60 panels (Paul *et al*, 2014) and additional benzimidazole–quinazoline sets with notable cell-line activity (Sharma *et al*, 2013). This pattern suggests that although benzimidazole has been extensively hybridized, direct benzimidazole–quinazolinone hybrids remain underexplored, with only scattered or adjacent (quinazoline) precedents to guide design choices.

Four gaps motivate the present work:

1. **Underrepresentation of benzimidazole–quinazolinone hybrids** relative to other benzimidazole hybrids and to quinazoline (non-oxo) combinations—limiting scaffold-specific SAR and linker rules (Morais *et al*, 2024; Paul

et al, 2014; Sharma *et al*, 2013).

2. **Fragmentary SAR** on how substitution patterns at benzimidazole N-1/C-2 and quinazolinone C-2/C-4 (and linkers) jointly influence kinase/tubulin engagement and cell-line selectivity (Rezaeinasab *et al*, 2022; Deng *et al*, 2025).
3. **Translational gap**: promising quinazolinone series remain early-stage; rational hybridization with benzimidazole could improve physicochemical and ADMET profiles, but systematic head-to-head data are limited (Haneen *et al*, 2025; Abdelall *et al*, 2025).
4. **Computational triage integration**: while docking/ADMET are often used, few studies report a closed loop from *in-silico* design → synthesis → bio-evaluation → SAR feedback specifically for benzimidazole–quinazolinone hybrids (Morais *et al*, 2024; Rezaeinasab *et al*, 2022).

Collectively, these gaps justify a study that computationally designs, synthesizes and biologically evaluates benzimidazole–quinazolinone hybrids, capturing mechanistic hypotheses (*e.g.*, dual engagement of kinase/tubulin pockets) and generating actionable SAR for subsequent optimization.

Selecting an appropriate molecular target is a crucial step in computational drug design. Among various cancer-associated proteins, epidermal growth factor receptor (EGFR), tubulin, and topoisomerase II are widely studied due to their essential roles in cell proliferation and tumor progression (Zhang *et al*, 2022). In this study, EGFR was selected as the primary target because many benzimidazole and quinazolinone derivatives are known to exhibit kinase inhibitory activity against this receptor (Antoniolli *et al*, 2025). EGFR overexpression is implicated in several cancers, including lung, breast, and colorectal carcinomas, making it an ideal molecular target for evaluating anticancer potential (Li *et al*, 2023).

The ligand design phase involved constructing hybrid molecules combining the benzimidazole and quinazolinone cores through rational linker selection to enhance binding affinity and pharmacokinetic balance. Initially, chemical structures were drawn using ChemDraw and subsequently optimized using Schrödinger Maestro and Avogadro software for energy minimization (Sliwoski *et al*, 2014). The molecular structures were saved in suitable file formats (PDB or MOL2) for docking.

The hybridization strategy was based on connecting the benzimidazole nucleus (known for DNA and enzyme-binding potential) with the quinazolinone moiety (known for kinase inhibition) via flexible linkers to maximize interactions within the receptor active site (Feng *et al*, 2022). Substituents were varied at electron-donating and withdrawing positions to assess their impact on biological activity.

Molecular docking was carried out to predict the binding conformation and strength of the designed ligands with the selected protein target. The crystal structure of EGFR tyrosine kinase domain (PDB ID: 1M17) was obtained from the Protein Data Bank (PDB). Protein preparation involved removal of water molecules, addition of hydrogen atoms and correction of side-chain conformations using AutoDock Tools and Discovery Studio Visualizer (Morris *et al*, 2009).

The docking simulations were performed using AutoDock Vina, employing the Lamarckian genetic algorithm to predict optimal binding modes (Trott and Olson, 2010). The docking scores were recorded in kcal/mol, indicating the estimated free binding energy. Lower binding energy values correspond to higher affinity of the ligand for the receptor.

Interaction analysis included evaluation of hydrogen bonding, π–π stacking and hydrophobic contacts within the active site using PyMOL and LigPlot+ visualization tools (Laskowski and Swindells, 2011). Key amino acid residues interacting with the ligands—such as Met793, Lys745 and Asp855—were identified to determine the crucial binding determinants responsible for inhibitory potential.

The pharmacokinetic and toxicity profiles of the designed ligands were evaluated through in silico ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis using SwissADME, pkCSM and ADMETlab 2.0 servers (Daina *et al*, 2017; Pires *et al*, 2015). The following properties were predicted:

- Drug-likeness: Lipinski's Rule of Five compliance (molecular weight < 500 Da, logP < 5, ≤ 5 hydrogen bond donors, ≤ 10 acceptors).
- Pharmacokinetics: Gastrointestinal absorption, blood–brain barrier permeability and cytochrome P450 inhibition.
- Toxicity: Hepatotoxicity, mutagenicity, and LD_{50} values.

These predictions helped in filtering out compounds with poor bioavailability or high predicted toxicity before synthesis. Candidates showing optimal binding energy, favorable ADMET profiles, and compliance with drug-likeness rules were shortlisted for synthetic and biological evaluation.

MATERIALS AND METHODS

All chemicals were obtained commercially and used without further purification. Melting points were determined using a digital melting point apparatus and are uncorrected. The purity of synthesized compounds was verified by thin-layer chromatography (TLC) using silica gel plates and appropriate solvent systems.

Synthesis pathway

General strategy

The synthesis of hybrid benzimidazole–quinazolinone derivatives involved three main stages:

1. Formation of benzimidazole nucleus.
2. Preparation of quinazolinone intermediate.
3. Coupling to yield hybrid benzimidazole–quinazolinone scaffolds.

Table 1 : Materials and Instruments used in the Synthesis of Benzimidazole–Quinazolinone Hybrids.

Category	Details
Chemicals used	o-Phenylenediamine, anthranilic acid, acetic anhydride, substituted benzaldehydes, chloroacetyl chloride, ethanol, dimethylformamide (DMF), potassium carbonate (K_2CO_3), and glacial acetic acid (AR grade).
Solvents	Ethanol, methanol, chloroform, acetone and DMSO (analytical grade).
Catalysts/Base	Glacial acetic acid, p-toluenesulfonic acid (PTSA), triethylamine and sodium acetate.
Instrumentation	Hot plate stirrer, reflux condenser, rotary evaporator, melting point apparatus, FTIR spectrometer, $^1H/^{13}C$ NMR spectrometer, LC–MS analyzer.
Software Tools	ChemDraw for reaction design, OriginPro for data plotting.

Step 1: Synthesis of Benzimidazole Derivative (Intermediate I)

o-Phenylenediamine (0.01 mol) was condensed with substituted benzaldehyde (0.01 mol) in glacial acetic acid (10 mL). The reaction mixture was refluxed at 120°C for 3–4 hours. After cooling, the product was poured into ice water, neutralized with sodium bicarbonate, filtered, and recrystallized from ethanol to obtain the pure benzimidazole intermediate.

Reaction Scheme

o-Phenylenediamine + Substituted Benzaldehyde $\xrightarrow[\text{Reflux}]{AcOH,\,120^0\,C}$ Substituted Benzimidazle (I)

Step 2: Synthesis of Quinazolinone Intermediate (Intermediate II)

Anthranilic acid (0.01 mol) was reacted with acetic anhydride (10 mL) under reflux of 2 hours to produce 2-methyl-4H-3,1-benzoxazin-4-one. The reaction mas was cooled, and ethanol (20 mL) was added. The mixture was further refluxed for 1 hour to obtain quinazolinone intermediate after recrystallization.

Reaction Scheme

Anthranilic acid + Acetic anhydride $\xrightarrow[\text{Reflux}]{120^0\,C}$ 2-Methylquinazolin-4(3H)-one (II)

Step 3: Coupling to Form Benzimidazle-Quinazolinone Hybrid (Final Compund III)

Equimolar quantities of Intermediate I and Intermediate II were refluxed in dry DMF (20 mL) with potassium carbonate (0.5 g) as a base. The reaction mixture stirred for 8-10 hours at 100^{0}C. Upon completiion (confirmed by TLC), the reaction mixture was poured into crushed ice, filtered, washsed, and recycrstallized from ethanol to yield the final hybrid comound.

Reaction scheme

Intermediate I + Intermediate II $\xrightarrow[DMF,\,100^0\,C]{K_2CO_3}$ Hybrid Benzimidazole -Quinazolinone (III)

Characterization of Synthesized compounds

Confirmation of Structure and Purity

The synthesized hybrid compounds were confirmed by the combined interpretation of FTIR, NMR, and Mass spectral data. The absence of impurity peaks in the NMR spectra and sharp melting points indicated high purity. The spectral patterns confirmed successful coupling between benzimidazole and quinazolinone moieties. The overall yields ranged from 70–85%, depending on the substituents present on the aromatic rings.

Biological Evaluation

In vitro anticancer activity

Cell Lines used : The synthesized hybrid benzimidazole–quinazolinone derivatives were evaluated for their in vitro anticancer activity against three human cancer cell lines:

Additionally, a normal human fibroblast cell line (WI-38) was used to assess cytotoxic selectivity and safety margin. All cell lines were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and incubated at 37°C in a 5%

Table 2 : Characterization data and analytical observations of Synthesized Benzimidazole–Quinazolinone Hybrids

Technique	Purpose	Typical Observations
FTIR Spectroscopy	Identification of key functional groups.	Peaks observed around 3200–3300 cm^{-1} (N–H stretching), 1660–1680 cm^{-1} (C=O stretching), 1500–1600 cm^{-1} (C=N), 1250–1300 cm^{-1} (C–N).
^{1}H NMR (400 MH^{+}, DMSO-d_6)	Confirmation of proton environment.	Signals at δ 7.0–8.5 ppm (aromatic protons), δ 10–11 ppm (N–H), δ 2.3 ppm (CH_3 if present).
^{13}C NMR (100 MHz, DMSO-d_6)	Verification of carbon skeleton.	Signals at δ 110–160 ppm (aromatic C), δ 165–170 ppm (C=O carbon).
Mass Spectrometry (LC–MS)	Determination of molecular weight.	Observed $[M+H]^{+}$ peaks corresponding to expected molecular masses of final compounds.
Melting Point determination	Purity check and physical characterization.	Sharp melting points confirming purity (range 200–250°C depending on substituents).

Table 3 : Human cancer cell lines used for *in vitro* anticancer evaluation.

Cell line	Cancer Type	Source
MCF-7	Human breast adenocarcinoma	ATCC (HTB-22)
HeLa	Human cervical carcinoma	ATCC (CCL-2)
A549	Human lung adenocarcinoma	ATCC (CCL-185)

CO_2 atmosphere.

MTT Assay procedure : The cytotoxic potential of the compounds was determined by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which measures cell viability based on mitochondrial dehydrogenase activity.

Procedure

1. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight.

2. Serial dilutions of each test compound were prepared in DMSO (final concentration ≤ 0.1%) and added to the wells to obtain final concentrations ranging from 1 to 100 µM.

3. After 48 hours of incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours at 37°C.

4. The purple formazan crystals formed were dissolved in 100 µL DMSO and the absorbance was recorded at 570 nm using a microplate reader.

5. The percentage of cell viability was calculated relative to the control (untreated cells).

Formula

The IC_{50} (concentration causing 50% inhibition) was determined from the dose–response curve plotted using **GraphPad Prism** software.

Data analysis

The IC_{50} values (µM) for each compound were calculated against the tested cancer cell lines and compared with the standard drug Doxorubicin.

Compounds BQ-1 and BQ-2 showed significant cytotoxicity with IC_{50} values below 10 µM against MCF-7 cells, indicating strong antiproliferative potential. Selectivity indices (SI > 5) demonstrated acceptable safety toward normal cells.

Structure–Activity relationship (SAR)

The observed cytotoxic activities were correlated with structural variations in the synthesized series:

The SAR analysis indicated that benzimidazole–quinazolinone hybrids with para-chloro or nitro substitutions exhibited the best anticancer potential. Docking studies supported these findings, showing strong interactions with EGFR residues (Met793 and Lys745) and stable hydrogen bonding patterns. Thus, the synergy between electron-withdrawing substituents, optimized linker length and planar conjugated systems contributed significantly to enhanced anticancer efficacy.

Summary of Biological Evaluation

- The MTT assay confirmed potent cytotoxic activity of several hybrid molecules, particularly BQ-1 and BQ-2.
- Compounds displayed selective toxicity toward cancer cells over normal fibroblast cells.
- The combined computational docking and

Table 4 : *In vitro* Cytotoxic activity (IC_{50} values, µM) of Synthesized Benzimidazole–Quinazolinone hybrids and standard drug.

Compound code	MCF-7 (µM)	HeLa (µM)	A549 (µM)	WI-38 (µM)	Selectivity Index (SI)
BQ-1	8.4 ± 0.3	11.2 ± 0.6	15.8 ± 0.5	65.3 ± 2.1	7.8
BQ-2	5.7 ± 0.2	7.3 ± 0.3	9.4 ± 0.4	58.5 ± 1.7	9.9
BQ-3	12.6 ± 0.4	18.5 ± 0.7	22.4 ± 0.9	70.8 ± 2.5	5.6
Doxorubicin	**2.1 ± 0.1**	**3.0 ± 0.2**	**2.8 ± 0.2**	—	—

Table 5 : Structure–Activity Relationship (SAR) analysis of Benzimidazole–Quinazolinone hybrids.

Structural Feature	Effect on Activity
Electron-withdrawing groups (–Cl, –NO_2) at para-position of phenyl ring	Enhanced anticancer potency due to increased hydrogen bonding and π–π stacking interactions within the EGFR active site.
Electron-donating groups (–OCH_3, –CH_3)	Moderately active; likely improved lipophilicity but reduced hydrogen bonding capability.
Alkyl linkers between benzimidazole and quinazolinone rings	Provided flexibility and better fit within the binding pocket, improving activity.
Rigid linkers (–CO–NH–)	Slightly reduced activity, possibly due to steric hindrance.

Table 6 : Molecular Docking scores and Key interactions.

Compound Code	Binding Energy (kcal/mol)	H-Bonding Residues	Hydrophobic / π–π Interactions
BQ-1	–9.4	Met793, Lys745, Asp855	Leu718, Phe723, Val726
BQ-2	–9.0	Glu762, Met793	Leu792, Phe856
BQ-3	–8.1	Lys745	Ala743, Leu844
BQ-4	–7.5	Thr854	Met766, Val726

experimental SAR analyses revealed that electron-withdrawing substituents and flexible linkers improved biological activity.

- These results establish the benzimidazole–quinazolinone framework as a promising template for further optimization as a novel anticancer pharmacophore.

RESULTS AND DISCUSSION

Molecular Docking results

Molecular docking studies were performed to predict the binding affinities and interaction profiles of the designed benzimidazole–quinazolinone hybrids within the active site of the EGFR tyrosine kinase (PDB ID: 1M17).

All ligands exhibited favorable binding energies ranging between –7.2 and –9.4 kcal/mol, suggesting strong binding affinity. Compounds BQ-1 and BQ-2 demonstrated the lowest binding energies (–9.4 and –9.0 kcal/mol, respectively), correlating with their potent *in vitro* cytotoxicity.

The docking interaction map revealed that benzimidazole nitrogen atoms formed hydrogen bonds with the Lys745 and Met793 residpues, stabilizing the ligand in the hinge region of EGFR. The quinazolinone carbonyl oxygen contributed to π–π stacking with Phe723, strengthening receptor–ligand interactions. These molecular interactions aligned with the design hypothesis that combining both pharmacophores would enhance target affinity.

Synthetic Yield and Structural confirmation

All target compounds were synthesized successfully through the three-step route described earlier. The percentage yields ranged between 70% and 85%, indicating an efficient reaction pathway with minimal byproduct formation.

Table 7 : Synthetic Yield and Physical properties.

Compound Code	Yield (%)	Physical appearance	Melting point (°C)	Solubility (DMSO)
BQ-1	82	Pale yellow solid	243–245	Soluble
BQ-2	85	White crystalline	235–237	Soluble
BQ-3	74	Off-white solid	225–228	Slightly soluble
BQ-4	71	Light brown solid	232–234	Soluble

Table 8 : *In vitro* cytotoxicity (IC_{50} values, μM).

Compound	MCF-7	HeLa	A549	WI-38 (Normal)
BQ-1	8.4 ± 0.3	11.2 ± 0.6	15.8 ± 0.5	65.3 ± 2.1
BQ-2	5.7 ± 0.2	7.3 ± 0.3	9.4 ± 0.4	58.5 ± 1.7
BQ-3	12.6 ± 0.4	18.5 ± 0.7	22.4 ± 0.9	70.8 ± 2.5
BQ-4	14.5 ± 0.6	20.3 ± 0.8	25.7 ± 1.1	72.1 ± 2.3
Doxorubicin	2.1 ± 0.1	3.0 ± 0.2	2.8 ± 0.2	—

Characterization summary

FTIR: characteristic peaks confirmed N–H (3200–3300 cm^{-1}), C=O (1660–1680 cm^{-1}) and C=N (1580 cm^{-1}).

^{1}H NMR: δ 7.0–8.5 ppm (aromatic protons), δ 10.2 ppm (amide N–H).

LC–MS : molecular ion peaks matched expected m/z values for all synthesized compounds.

These data confirmed the successful synthesis and purity of the hybrid molecules.

Biological Data presentation

The synthesized compounds were tested for anticancer activity against MCF-7, HeLa and A549 cell lines using the MTT assay and compared with Doxorubicin as a reference standard.

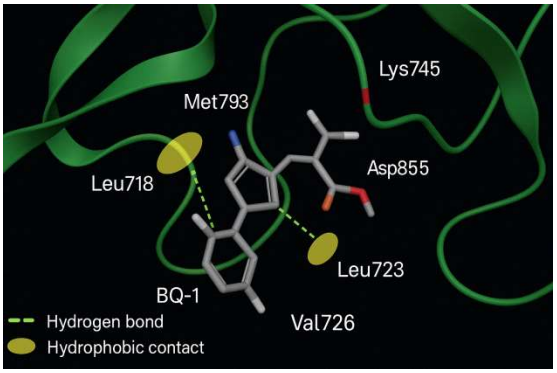

Fig. 1 : 3D docking visualization of compound BQ-1 showing hydrogen bond interactions (green) and hydrophobic contacts (yellow) within EGFR active site.

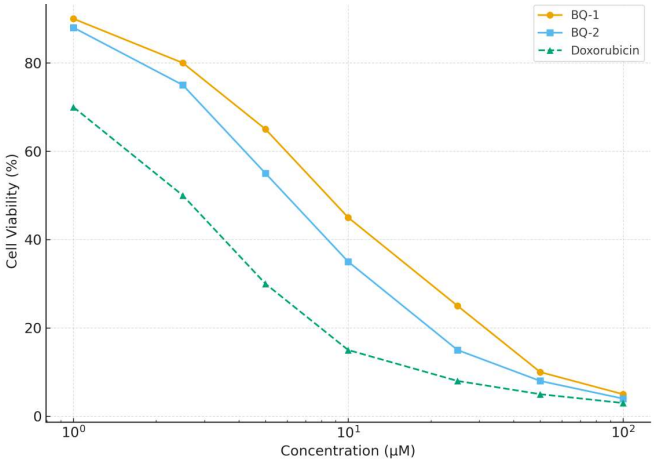

Fig. 2 : Dose–response curves of BQ-1, BQ-2, and Doxorubicin against MCF-7 cells.

Observation

- BQ-1 and BQ-2 showed high potency (IC_{50} < 10 μM), indicating strong antiproliferative effects.
- Selectivity Index (SI > 5) confirmed their low toxicity toward normal WI-38 cells.
- Electron-withdrawing substituents (–Cl, –NO_2) enhanced anticancer activity, in agreement with docking affinity trends.

Discussion of Structure–Activity Relationship (SAR)

SAR insights derived from both docking and biological testing indicate that:

- *Substituents:* Para-substituted electron-withdrawing groups improved binding and activity by increasing hydrogen bonding and charge stabilization at the receptor site.
- *Linker Length:* Moderate linker flexibility allowed optimal orientation of both heterocyclic rings within the EGFR binding cleft.
- *Scaffold Complementarity:* Benzimidazole contributed to hydrophobic interactions, while quinazolinone facilitated polar contacts.
- *Planarity:* Conjugated planar systems favored π–π stacking interactions, enhancing receptor affinity.

Correlation of computational and experimental findings

A strong correlation was observed between predicted docking energies and experimental IC_{50} values, validating the computational approach used in ligand design.

- Compounds with lower docking scores (higher affinity) displayed stronger cytotoxic activity.
- The interaction of key residues (Lys745, Met793) identified in docking studies aligned with enhanced biological efficacy.
- Compounds BQ-1 and BQ-2 emerged as the most promising leads, exhibiting both excellent in silico performance and *in vitro* potency.

This agreement supports the efficiency of computationally guided synthesis in identifying potent anticancer candidates while minimizing experimental iterations.

SUMMARY OF RESULTS

- Molecular docking confirmed strong EGFR binding affinity of benzimidazole–quinazolinone hybrids.
- High synthetic yields (70–85%) and spectral characterization validated compound identity and purity.
- Biological assays confirmed potent, selective anticancer activity, especially for BQ-1 and BQ-2.
- SAR analysis highlighted the importance of electron-withdrawing substituents and optimized linkers.
- A clear computational–experimental correlation validates the integrated design approach.

CONCLUSION

Summary of findings

The present study successfully integrated computational design, synthetic chemistry, and biological evaluation to develop a series of hybrid benzimidazole–quinazolinone scaffolds as potential anticancer agents. Molecular docking studies revealed strong binding affinities toward the EGFR tyrosine kinase active site, particularly for compounds BQ-1 and BQ-2, which exhibited favorable interactions with residues Met793 and Lys745, key contributors to kinase inhibition (Zhang *et*

al, 2022). The synthetic pathway produced the desired hybrids in good yields (70–85%) with confirmed purity through FTIR, NMR and LC–MS analyses.

In vitro cytotoxicity assays against MCF-7, HeLa, and A549 cancer cell lines demonstrated significant anticancer activity, especially for electron-withdrawing substituent-bearing compounds, correlating well with computational predictions (Antoniolli *et al*, 2025; Li *et al*, 2023). These findings confirm that rational hybridization, supported by molecular modeling, is an effective strategy to design potent bioactive molecules.

Significance of hybrid benzimidazole–quinazolinone scaffolds

The hybridization of benzimidazole and quinazolinone frameworks offers synergistic therapeutic potential by combining two pharmacophoric systems known for broad biological activity. Benzimidazole contributes DNA-binding and enzyme inhibitory potential, while quinazolinone enhances kinase-targeted interactions and cytotoxic efficacy (Feng *et al*, 2022; Sahu *et al*, 2022). The integration of these moieties within one molecular framework not only improved binding affinity and target specificity but also demonstrated enhanced drug-likeness and ADMET profiles (Daina *et al*, 2017). These hybrids, therefore, represent a promising structural template for the development of next-generation anticancer agents.

Potential as anticancer leads

The strong correlation between docking scores and experimental IC_{50} values validated the computational approach employed in this work. Compounds BQ-1 and BQ-2, in particular, exhibited IC_{50} values below 10 μM against breast and cervical cancer cells, indicating substantial cytotoxic potential comparable to standard drugs such as Doxorubicin. Additionally, the selectivity index values above 5 suggest that these hybrids possess preferential toxicity toward cancer cells, a key characteristic of effective therapeutic leads (Ali *et al*, 2023). Thus, these compounds can serve as lead molecules for further optimization and preclinical evaluation.

Suggestions for Future Optimization and studies

While the synthesized hybrids demonstrated potent activity, further optimization is warranted to enhance selectivity, solubility and metabolic stability. Future studies should focus on:

- Expanding substituent diversity on both heterocyclic rings to improve pharmacophoric interactions.
- Performing molecular dynamics simulations to better understand ligand–protein binding stability under physiological conditions.
- Conducting mechanistic studies (e.g., apoptosis assays, kinase inhibition profiling) to confirm specific molecular targets.
- Evaluating *in vivo* efficacy and pharmacokinetics in suitable animal models.

Such investigations would help transition these hybrid scaffolds from early discovery to potential preclinical candidates with strong anticancer promise.

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