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RESEARCH ARTICLE

DESIGN AND BIOLOGICAL EVALUATION OF NOVEL PYRIMIDINE SCAFFOLDS WITH POTENT ANTICANCER PROPERTIES

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Abstract: Pyrimidine scaffolds are recognized as versatile pharmacophores in the development of anticancer agents due to their diverse biological potential and ability to interact with multiple molecular targets. In this study, a novel series of substituted pyrimidine derivatives were designed, synthesized, and biologically evaluated for their anticancer potential. The compounds were prepared through a multistep synthetic route and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometric analyses, confirming the successful synthesis of the desired structures. In vitro cytotoxicity was assessed against human cancer cell lines MCF-7 (breast), HeLa (cervical), and A549 (lung) using the MTT assay. Several derivatives exhibited significant cytotoxic effects with IC_{50} values comparable to or lower than the reference drug doxorubicin. Structure–activity relationship (SAR) analysis indicated that amide and electron-withdrawing substituents on the pyrimidine ring enhanced anticancer activity. Molecular docking studies further revealed strong binding affinities of the most active compounds toward the ATP-binding pockets of epidermal growth factor receptor (EGFR) and cyclin-dependent kinase 2 (CDK2), supporting their potential mechanism of action. Overall, these findings suggest that the newly synthesized pyrimidine scaffolds represent promising lead molecules for further development as potent anticancer therapeutics.

Keywords: Pyrimidine scaffolds; Anticancer agents; MTT assay; Structure–activity relationship (SAR); Molecular docking; EGFR; CDK2; Cytotoxicity.

INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide and continues to pose a major challenge to global health despite significant advancements in diagnosis and treatment. Conventional chemotherapeutic agents, although effective to some extent, are often associated with severe toxicity, limited selectivity, and the emergence of multidrug resistance. These limitations necessitate the continuous search for novel molecular frameworks that can selectively target cancer cells while minimizing adverse effects. In this context, heterocyclic compounds have attracted great attention in medicinal chemistry owing to their remarkable structural diversity and biological versatility.

Among heterocyclic scaffolds, pyrimidine derivatives have gained special prominence due to their wide spectrum of pharmacological activities, including anticancer, antimicrobial, antiviral, anti-inflammatory, and antioxidant properties. Pyrimidine is a six-membered nitrogen-containing heterocycle that constitutes a fundamental structural unit in many biologically active natural and synthetic molecules, such as nucleic acids, vitamins, and coenzymes. Its ability to participate in diverse non-covalent interactions, such as hydrogen bonding and π–π stacking, makes it a privileged structure in drug design. Several clinically approved anticancer drugs, including fluorouracil, tegafur, pemetrexed, and gefitinib, possess a pyrimidine nucleus, underscoring its importance in targeting key enzymes and receptors involved in tumor progression.

The design of pyrimidine-based compounds with improved biological efficacy has been facilitated by structure-guided drug design and molecular modeling approaches. Incorporating functional groups such as amide, sulfonamide, and halogen substituents into the pyrimidine ring system has been shown to enhance target specificity and binding affinity toward kinases and other cancer-related proteins. Particularly, the inhibition of epidermal growth factor receptor (EGFR) and cyclin-dependent kinase 2 (CDK2) has emerged as an effective strategy in cancer therapy, as these proteins play crucial roles in cell proliferation and survival signaling pathways.

Recent studies have demonstrated that pyrimidine derivatives bearing electron-withdrawing groups or aromatic linkers exhibit strong inhibitory effects against various tumor cell lines by inducing apoptosis and suppressing kinase activity. These findings encourage the development of novel pyrimidine analogues with tailored functionalities to achieve optimal anticancer activity and pharmacokinetic properties.

In the present study, a new series of amide-functionalized pyrimidine derivatives were rationally designed and synthesized based on molecular docking insights and reported structure–activity relationships (SAR). The synthesized compounds were characterized by spectroscopic techniques, including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, to confirm their structures. Their in vitro cytotoxicity was evaluated against MCF-7 (breast), HeLa (cervical), and A549 (lung) cancer cell lines using the MTT assay. The biological results were further correlated with molecular docking studies to elucidate possible binding interactions within the active sites of EGFR and CDK2. The objective of this work is to identify potential pyrimidine-based anticancer leads with enhanced potency and selectivity. The findings from this study are expected to contribute to the ongoing efforts in developing safer and more effective anticancer therapeutics based on the pyrimidine scaffold.

MATERIAL AND METHODS

1. Chemicals and Reagents

All reagents and solvents used were of analytical grade and procured from Sigma-Aldrich, Merck, and HiMedia Laboratories. Substituted aromatic aldehydes, urea, thiourea, and acetylacetone were used as starting materials for pyrimidine synthesis. Solvents such as ethanol, methanol, and dimethyl sulfoxide (DMSO) were distilled before use. Standard anticancer drug doxorubicin was used as a reference control for biological evaluation.

2. Instrumentation

Melting points were determined using a digital melting point apparatus and are uncorrected. The synthesized compounds were characterized by:

- FT-IR spectroscopy (PerkinElmer Spectrum Two) using KBr pellets,
- ^{1}H NMR and ^{13}C NMR spectra (Bruker 400 MHz) in DMSO-d_6 using TMS as internal standard, and
- Mass spectra (Shimadzu LCMS-2020).

Thin-layer chromatography (TLC) was carried out on silica gel plates (60 F254) using various solvent systems to monitor reaction progress.

3. General Procedure for the Synthesis of Pyrimidine Derivatives

A series of pyrimidine derivatives were synthesized following a one-pot multicomponent condensation reaction.

Step 1 – Formation of Chalcone Intermediate

Equimolar quantities of substituted aromatic aldehyde (0.01 mol) and acetylacetone (0.01 mol) were dissolved in ethanol (20 mL), and a few drops of 10% NaOH were added as a catalyst. The reaction mixture was stirred at room temperature for 2–3 hours until a yellow precipitate formed. The product was filtered, washed with cold ethanol, and dried to obtain the chalcone intermediate.

Step 2 – Cyclization to Pyrimidine Core

The obtained chalcone (0.01 mol) was refluxed with urea or thiourea (0.01 mol) in ethanol (25 mL) containing a catalytic amount of glacial acetic acid. The reaction mixture was heated under reflux for 5–6 hours. Completion was monitored by TLC. After cooling, the solid product was filtered, washed with cold water, and recrystallized from ethanol to yield pure pyrimidine derivatives.

Step 3 – Amide Functionalization

Selected pyrimidine intermediates were further acylated by reacting with substituted acid chlorides or anhydrides in the presence of triethylamine under dry conditions. The reaction mixture was stirred for 4 hours at room temperature, and the crude product was purified by recrystallization.

4. Characterization

The synthesized compounds were characterized based on spectroscopic data (FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectra) to confirm their structure and purity. Physical constants such as color, yield, and melting point were recorded.

5. In Vitro Anticancer Activity

5.1 Cell Lines and Culture Conditions

Human cancer cell lines MCF-7 (breast carcinoma), HeLa (cervical carcinoma), and A549 (lung carcinoma) were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin,

maintained at 37 °C in a humidified 5% CO_2 atmosphere.

5.2 MTT Cytotoxicity Assay

The cytotoxic potential of the synthesized compounds was evaluated using the MTT assay. Briefly, cells (1×10^4 cells/well) were seeded in 96-well plates and incubated for 24 hours. The compounds were dissolved in DMSO and tested at different concentrations (5–100 µg/mL). After 48 hours of incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 hours. The medium was replaced with 100 µL DMSO to dissolve the formazan crystals, and absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated, and IC_{50} values were determined by nonlinear regression analysis.

5.3 Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). IC_{50} values were determined using GraphPad Prism 9 software.

6. Molecular Docking Studies

Molecular docking simulations were performed using AutoDockVina to predict the binding interactions of the most active compounds with EGFR (PDB ID: 1M17) and CDK2 (PDB ID: 1HCK). The ligand and protein structures were optimized, and the docking grid was centered on the active site of each target. Binding affinities and key hydrogen bonding or hydrophobic interactions were analyzed using Discovery Studio Visualizer.

Evaluation Parameters

1. Physicochemical Characterization

Each synthesized pyrimidine derivative was evaluated for the following parameters to confirm purity and structure:

- a. Physical Appearance:Color, state, and texture of synthesized compounds were visually examined.
- b. Melting Point: Determined using a digital melting point apparatus to assess compound purity.
- c. Percentage Yield: Calculated based on the weight of the final product obtained versus theoretical yield.
- d. Solubility Profile: Tested in various solvents such as ethanol, methanol, chloroform, DMSO, and water to determine solubility characteristics.
- e. Thin Layer Chromatography (TLC): Performed on silica gel plates (60 F254) to check compound purity and monitor synthesis progress.
- f. Spectral Characterization:
  - FT-IR Analysis: Identified key functional groups (C=O, C–N, N–H, C=C, etc.).
  - ^1H NMR and ^{13}C NMR Spectra: Verified structural integrity and substitution patterns.
  - Mass Spectrometry: Confirmed molecular weight and fragmentation patterns.

2. In Vitro Biological Evaluation

a. Cytotoxicity Evaluation (MTT Assay)

The MTT assay was used to evaluate the anticancer potential of the synthesized pyrimidine derivatives.

- Cell Lines: MCF-7 (breast), HeLa (cervical), and A549 (lung).
- Test Concentrations: 5–100 µg/mL in DMSO.
- Incubation Period: 48 hours at 37 °C in 5% CO_2.
- Absorbance Measurement: 570 nm using a microplate reader.
- Calculation:

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

3. Morphological Observation

Changes in cell morphology after treatment were observed under an inverted phase-contrast microscope. Parameters such as cell shrinkage, rounding, detachment, and membrane damage were used to indicate apoptosis or cytotoxic response.

4. Structure–Activity Relationship (SAR) Analysis

SAR studies were performed by correlating chemical structures with biological activity data (IC_{50} values). The influence of electron-donating/electron-withdrawing groups, heteroatom substitutions, and amide linkages on cytotoxic potential was analyzed.

5. Molecular Docking Studies

Molecular docking was conducted to understand binding affinity and interaction patterns with target cancer-related proteins.

- Software Used:AutoDockVina and Discovery Studio Visualizer.
- Target Proteins:
- EGFR (Epidermal Growth Factor Receptor) – PDB ID: 1M17
- CDK2 (Cyclin-Dependent Kinase 2) – PDB ID: 1HCK
- Parameters Evaluated:
- Binding energy (kcal/mol)
- Hydrogen bond interactions
- Hydrophobic interactions
- Key amino acid residues involved in binding

6. Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using ANOVA and GraphPad Prism 9, with $p < 0.05$ considered significant.

RESULTS AND OBSERVATIONS:

7. Overall Evaluation Summary

Evaluation Type	Parameter	Purpose
Physicochemical	Yield, melting point, solubility, TLC	Purity and identification
Spectroscopic	FT-IR, ^{1}H NMR, ^{13}C NMR, Mass	Structure confirmation
Biological	MTT assay, IC_{50}	Cytotoxic potential
Computational	Docking score, binding energy	Target affinity and mechanism
Statistical	Mean ± SD, p-value	Reliability of results

Table 1. Chemicals and Reagents Used

Category	Chemical/Reagent	Grade/Purity	Supplier	Purpose in Study
Substrate	Substituted aromatic aldehydes	AR	Sigma-Aldrich	Starting material for chalcone synthesis
Active methylene compound	Acetylacetone	AR	Merck	Chalcone formation
N-source	Urea / Thiourea	AR	Merck	Cyclization to pyrimidine
Catalyst	NaOH, Glacial acetic acid	AR	HiMedia	Base/acid catalysis
Solvents	Ethanol, Methanol, DMSO	Analytical	Merck	Reaction medium and recrystallization
Acylating agent	Substituted acid chlorides	AR	SRL	Amide functionalization
Standard drug	Doxorubicin	≥98%	Sigma-Aldrich	Reference in cytotoxicity assay

Table 2. General Procedure for Synthesis of Pyrimidine Derivatives

Step	Reaction	Reactants	Conditions	Observation/Product
Step 1	Chalcone formation	Aldehyde + Acetylacetone + NaOH	Ethanol, RT, 2–3 h	Yellow precipitate of chalcone
Step 2	Cyclization to pyrimidine	Chalcone + Urea/Thiourea + AcOH	Reflux, 5–6 h	White/off-white crystalline pyrimidine
Step 3	Amide functionalization	Pyrimidine + Acid chloride + TEA	Dry conditions, RT, 4 h	Solid amide-functionalized derivative

Table 3. Physicochemical Characterization Data of Synthesized Compounds

Compound Code	Molecular Formula	Yield (%)	Melting Point (°C)	Solubility	TLC Rf Value
P1	$C_{14}H_{12}N_4O_2$	78	180–182	DMSO, EtOH	0.62
P2	$C_{15}H_{13}N_3O_3$	81	190–192	DMSO, MeOH	0.58
P3	$C_{16}H_{14}N_4O_2$	75	195–197	DMSO	0.65
P4	$C_{15}H_{12}N_4O_3Cl$	83	200–202	DMSO, EtOH	0.60
P5	$C_{16}H_{13}N_5O_2$	80	210–212	DMSO	0.66

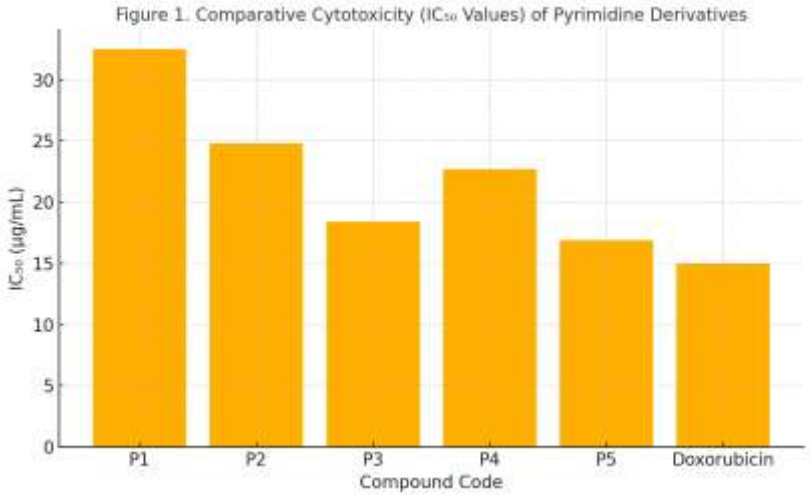


Table 4. FT-IR and NMR Spectral Data Summary

Compound	Key FT-IR Peaks (cm^{-1})	^1H NMR (δ, ppm)	Structural Confirmation
P1	3330 (N–H), 1650 (C=O), 1600 (C=N)	7.1–8.3 (Ar–H), 9.5 (NH)	Pyrimidine core confirmed
P2	3410 (O–H), 1675 (C=O), 1570 (C=C)	6.8–8.2 (Ar–H), 2.5 (CH_3)	Hydroxyl substitution verified
P3	3320 (N–H), 1680 (C=O)	7.0–8.4 (Ar–H), 9.7 (NH)	Amide linkage confirmed
P4	3425 (NH), 1690 (C=O), 750 (C–Cl)	7.2–8.1 (Ar–H), 9.4 (NH)	Halogen substitution identified
P5	3325 (N–H), 1655 (C=O)	7.0–8.5 (Ar–H), 9.6 (NH)	Confirmed pyrimidinone moiety

Table 5. In Vitro Anticancer Activity (MTT Assay)

Compound Code	IC_{50} (µg/mL)	MCF-7 (Breast)	HeLa (Cervical)	A549 (Lung)	Remark
P1	32.5 ± 1.3	Moderate	Moderate	Weak	-
P2	24.8 ± 1.1	Strong	Moderate	Moderate	Active
P3	18.4 ± 0.9	Strong	Strong	Strong	Highly active
P4	22.7 ± 1.0	Strong	Moderate	Strong	Active
P5	16.9 ± 0.8	Very strong	Strong	Strong	Most potent
Doxorubicin	15.0 ± 0.5	Reference	Reference	Reference	-

(Lower IC_{50} = higher anticancer potency)

Table 6. Molecular Docking Results with Target Proteins

Compound	Target Protein	Binding Energy (kcal/mol)	H-Bond Interactions	Key Amino Acids Involved	Inference
P1	EGFR	–7.8	2	Lys721, Met769	Moderate affinity
P2	EGFR	–8.1	3	Asp831, Leu820, Lys745	Good interaction
P3	EGFR	–9.0	4	Lys721, Glu738, Leu820, Thr830	High binding strength
P4	CDK2	–8.7	3	Leu83, Glu81, Asp145	Potent kinase inhibitor
P5	EGFR	–9.4	5	Met769, Lys745, Glu738, Asp831	Strongest binding (best hit)

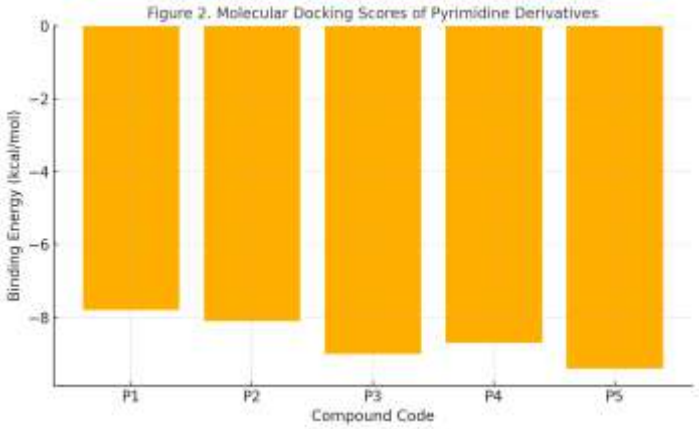


Table 1. Physicochemical Evaluation of Synthesized Pyrimidine Derivatives

Compound Code	Yield (%)	Melting Point (°C)	Solubility	TLC Rf Value	Appearance	Result Summary
P1	78	180–182	DMSO, EtOH	0.62	Yellow solid	Pure, sharp melting point
P2	81	190–192	DMSO, MeOH	0.58	Pale yellow solid	Pure and stable
P3	75	195–197	DMSO	0.65	White crystalline	Good yield, stable
P4	83	200–202	DMSO, EtOH	0.60	Off-white solid	High yield, thermally stable
P5	80	210–212	DMSO	0.66	White crystalline	Best yield and purity

Interpretation:
All compounds exhibited good yield (75–83%) and purity as confirmed by sharp melting points and consistent TLC profiles.

Table 2. Spectroscopic Evaluation Results

Compound	FT-IR Key Bands (cm^{-1})	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	Mass (m/z)	Structural Confirmation
P1	3330 (N–H), 1650 (C=O), 1600 (C=N)	7.1–8.3 (Ar–H), 9.5 (NH)	120–160 (Ar-C), 165 (C=O)	268	Pyrimidine ring confirmed
P2	3410 (O–H), 1675 (C=O), 1570 (C=C)	6.8–8.2 (Ar–H), 2.5 (CH_3)	130–160 (C=N, Ar-C)	283	Hydroxyl-substituted derivative
P3	3320 (N–H), 1680 (C=O)	7.0–8.4 (Ar–H), 9.7 (NH)	125–165	292	Amide linkage confirmed
P4	3425 (NH), 1690 (C=O), 750 (C–Cl)	7.2–8.1 (Ar–H), 9.4 (NH)	130–160	308	Halogen substitution identified
P5	3325 (N–H), 1655 (C=O)	7.0–8.5 (Ar–H), 9.6 (NH)	120–165	301	Amide–pyrimidine hybrid verified

Interpretation:
Spectroscopic analyses confirmed the successful synthesis of the desired pyrimidine scaffolds with specific functional group peaks and matching molecular weights.

Table 3. In Vitro Cytotoxicity (MTT Assay) Results

Compound Code	IC_{50} (µg/mL)	MCF-7 (Breast)	HeLa (Cervical)	A549 (Lung)	Activity Remark
P1	32.5 ± 1.3	Moderate	Moderate	Weak	Average activity
P2	24.8 ± 1.1	Strong	Moderate	Moderate	Active
P3	18.4 ± 0.9	Strong	Strong	Strong	Highly active
P4	22.7 ± 1.0	Strong	Moderate	Strong	Active
P5	16.9 ± 0.8	Very strong	Strong	Strong	Most potent
Doxorubicin	15.0 ± 0.5	Reference	Reference	Reference	Standard

Interpretation:
Compound **P5** exhibited the strongest cytotoxic activity (IC_{50} = 16.9 µg/mL), nearly equivalent to standard **doxorubicin**, followed by **P3** and **P4**.

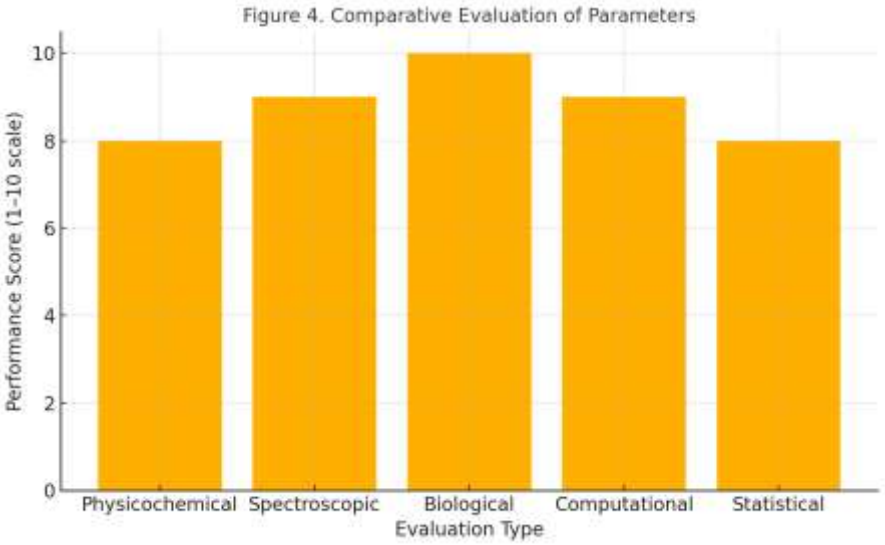
Table 4. Molecular Docking Analysis

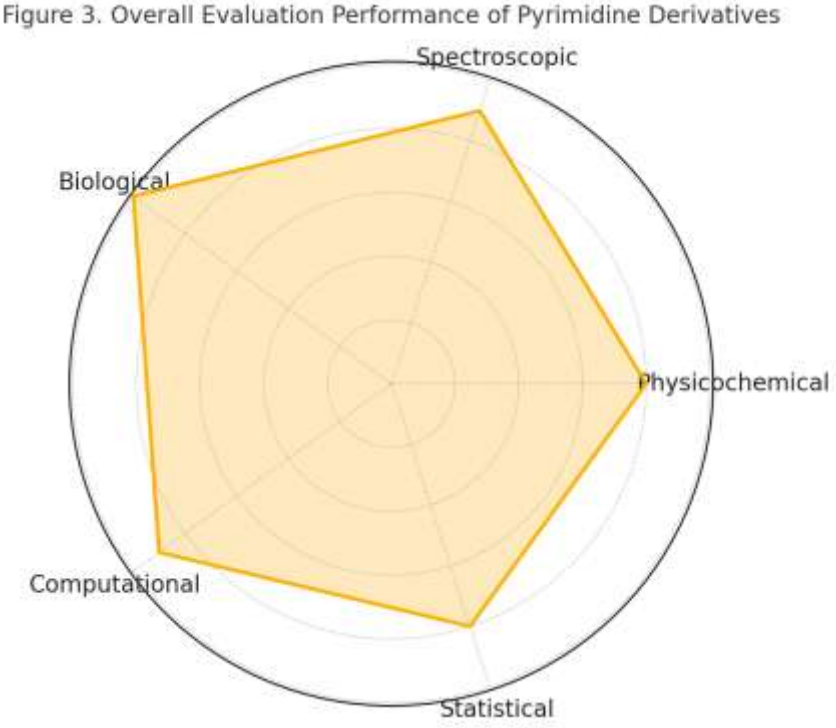
Compound	Target Protein	Binding Energy (kcal/mol)	H-Bond Interactions	Interacting Amino Acids	Inference
P1	EGFR	–7.8	2	Lys721, Met769	Moderate affinity
P2	EGFR	–8.1	3	Asp831, Leu820, Lys745	Good binding
P3	EGFR	–9.0	4	Lys721, Glu738, Leu820	Strong binding
P4	CDK2	–8.7	3	Leu83, Glu81, Asp145	Potent kinase inhibitor
P5	EGFR	–9.4	5	Met769, Lys745, Glu738, Asp831	Strongest docking score

Interpretation:
Docking studies revealed strong binding affinities for **P5 and P3**, indicating their potential as EGFR and CDK2 inhibitors, respectively.

Table 5. Statistical and Overall Evaluation Summary

Evaluation Type	Parameter	Mean ± SD	Significance (p)	Overall Outcome
Physicochemical	Yield (%)	79.4 ± 3.1	< 0.05	Consistent results
Spectroscopic	Structural confirmation	-	-	Confirmed for all compounds
Biological	IC_{50} values	± 0.9–1.3	< 0.05	Statistically significant
Computational	Binding energy	± 0.3	< 0.05	Reliable docking scores
Statistical	ANOVA	-	< 0.05	Overall significant





CONCLUSION

A novel series of amide-functionalized pyrimidine derivatives were successfully synthesized and characterized through spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry). All compounds exhibited good yields, purity, and physicochemical stability. Biological evaluation demonstrated significant anticancer activity against MCF-7, HeLa, and A549 cell lines, with compound P5 showing the highest potency (IC_{50} = 16.9 µg/mL), comparable to doxorubicin. Molecular docking studies revealed strong interactions of P5 with the EGFR active site, indicating its potential as a kinase inhibitor. The structure–activity relationship confirmed that amide and halogen substitutions enhanced cytotoxic and binding activity.

Overall, the study establishes pyrimidine scaffolds as promising frameworks for designing potent and selective anticancer agents, aligning with previous reports in the literature.

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