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Structure-Activity Relationship (SAR) and Mechanistic Insights of Novel Heterocyclic Derivatives with Promising Anticancer, Antimicrobial, and Anti-inflammatory Activities

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Abstract
In the ongoing pursuit of novel bioactive heterocycles, the present study reports the synthesis and comprehensive characterization of structurally diverse compounds, including 3,4-dihydro-2H-pyrrolo[3,4-b]quinazoline, 2-(4-bromo-2-chlorophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridine, and 5-(phenylthio)-2-methyl-1,3,4-thiadiazole. Characterization using advanced spectroscopic techniques (NMR, IR, MS) confirmed their molecular frameworks. Detailed structure-activity relationship (SAR) analysis demonstrated key structural features driving potent biological activities. The synthesized derivatives exhibited significant cytotoxic effects against MCF-7 and A549 cancer cell lines, potent kinase inhibition, broad-spectrum antibacterial activity, and marked anti-inflammatory effects. Mechanistic insights derived from in silico docking and kinase binding profiles further clarified the probable modes of action. This study emphasizes the therapeutic potential of these scaffolds and provides a foundation for future medicinal chemistry optimization.

Keywords: e.g., *Heterocyclic compounds, Kinase inhibition, Antimicrobial activity, Anti-inflammatory, Molecular docking, SAR*

1. Introduction

Heterocyclic compounds form the core of a vast majority of therapeutic agents due to their wide-ranging pharmacological activities and ability to interact with biological targets. The structural flexibility and chemical stability of heterocycles make them valuable scaffolds in drug discovery. Their prominence in medicinal chemistry stems from the ease with which they can be modified to enhance potency, selectivity, and pharmacokinetic properties (Patel et al., 2023). Among the most extensively studied heterocycles are quinazoline, imidazopyridine, and thiadiazole derivatives. Quinazolines have demonstrated potent activity against tyrosine kinases, making them effective in the treatment of cancers such as breast and lung carcinomas (Thakur et al., 2023). The imidazopyridine ring system, on the other hand, has been successfully incorporated into various kinase inhibitors, as well as antimicrobial and CNS-targeting agents (Zhou et al., 2024). Thiadiazoles, particularly the 1,3,4-thiadiazole moiety, have been reported for their broad-spectrum biological activities including antibacterial, antifungal, anti-inflammatory, and anticancer potentials (Hasan et al., 2023). Heterocyclic compounds, due to their drug-likeness and target versatility, provide an ideal platform for developing next-generation therapeutics (Mehta & Bhatt, 2023). The success of drugs such as gefitinib, erlotinib, and sorafenibquinazoline-based tyrosine kinase inhibitors has inspired researchers to investigate related analogs with improved activity and safety profiles (Roy et al., 2023).
Structure-Activity Relationship (SAR) studies play a pivotal role in understanding how structural modifications influence biological responses. For instance, electron-withdrawing groups on the quinazoline ring have been found to enhance antiproliferative effects by improving binding with ATP pockets of kinases. In thiadiazole derivatives, the presence of aryl or alkylthio substituents is associated with increased antimicrobial action (Das & Sharma, 2024). Mechanistic insights, particularly through molecular docking and ADMET profiling, allow medicinal chemists to predict and optimize drug-target interactions. Computational modeling aids in identifying favorable conformations, potential binding sites, and pharmacophoric elements essential for receptor engagement (Lee et al., 2024). Recent advances in AI-driven drug design have further accelerated the identification of bioactive heterocycles by enabling rapid virtual screening of large chemical libraries (Wang et al., 2024).

Furthermore, the integration of spectroscopic techniques, such as Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (IR), and Mass Spectrometry (MS), provides robust evidence for the structural integrity of synthesized molecules. These tools not only confirm purity and structure but also provide insights into functional groups that may influence pharmacological activity (Yadav et al., 2023). The biological efficacy of these heterocycles often extends beyond monotherapy. Heterocyclic frameworks can serve as pharmacophores for hybrid drug development, wherein two active moieties are combined to yield multifunctional agents with synergistic effects. This approach is particularly relevant in oncology, where heterocycle-based kinase inhibitors can be coupled with DNA-damaging agents or immunomodulators for enhanced treatment outcomes (Fernandez et al., 2024).

In recent years, green chemistry approaches have also gained traction in the synthesis of heterocycles, emphasizing the use of eco-friendly solvents, microwave-assisted synthesis, and recyclable catalysts. Such methods not only reduce environmental impact but also improve reaction efficiency and yield (Sharma et al., 2023). The current study explores novel derivatives of quinazoline, imidazopyridine, and thiadiazole, highlighting their synthesis, characterization, SAR, and mechanistic actions against key disease targets. By combining in vitro assays with in silicomodeling, this research aims to bridge the gap between bench chemistry and biological application, thus contributing to the evolving landscape of heterocyclic drug discovery.

2. Materials and Methods

2.1. Chemical Synthesis of Heterocyclic Derivatives

All reagents and solvents were purchased from Sigma-Aldrich or Merck and used without further purification unless otherwise specified. The synthesis of heterocyclic compounds was conducted using conventional condensation, cyclization, and halogenation techniques.

- **Pyrrolo[3,4-b]quinazoline derivatives** (Sharma et al., 2022).
- **Imidazo[4,5-b]pyridine derivatives** (Zhou et al., 2024).
- **Thiadiazole derivatives** (Hasan et al., 2023).

2.2. Spectroscopic Characterization

The synthesized compounds were characterized using standard analytical methods(Yadav et al., 2023).These methods ensured accurate structural elucidation and verified the substitution patterns on the heterocyclic rings

- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** ^1H and ^13C NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d6 or $CDCl_3$ as solvents.
- **Infrared (IR) Spectroscopy:** IR spectra were acquired using a Shimadzu FTIR-8400 spectrophotometer with samples in KBr pellets.
- **Mass Spectrometry (MS):** Mass spectra were recorded using LC-MS/MS (Agilent 6545 Q-TOF) for molecular ion confirmation.

2.3. Biological Activity Evaluation

2.3.1. Anticancer Assay

The cytotoxic potential of synthesized compounds was evaluated using the **MTT assay** against human cancer cell lines MCF-7 (breast cancer) and A549 (lung cancer). Cells were cultured in DMEM supplemented with 10% FBS and incubated with test compounds (1–100 µM) for 48 hours. Absorbance was measured at 570 nm to determine IC_{50} values (Roy et al., 2023).

2.3.2. Antimicrobial Screening

Antibacterial activity was tested using the **agar well diffusion method** against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. Zones of inhibition were measured, and MIC values were determined using the broth dilution method as per CLSI guidelines (Singh et al., 2022).

2.3.3. Anti-inflammatory Activity

The anti-inflammatory potential was assessed via the **protein denaturation method**, where the percentage inhibition of heat-induced albumin denaturation was calculated. Diclofenac sodium was used as the standard (Hasan et al., 2023).

2.4. In Silico Studies

Molecular docking was conducted using AutoDockVina to explore ligand-protein interactions with target kinases (EGFR and VEGFR), bacterial enzymes (DNA gyrase), and inflammatory mediators (COX-2). Ligand structures were energy-minimized, and docking scores and interaction profiles were analyzed with PyMOL and Discovery Studio Visualizer (Lee et al., 2024).

3. Results and Discussion

3.1 Synthesis of Novel Heterocyclic Compounds

A focused library of novel heterocyclic compounds was synthesized employing diverse organic synthetic strategies, including condensation, cyclization, halogenation, and thiation reactions. These methods facilitated the efficient assembly of core heterocyclic frameworks with potential biological relevance.

- **Synthesis of 3,4-Dihydro-2H-pyrrolo[3,4-b]quinazoline Derivatives:** These compounds were synthesized via intramolecular cyclization of o-aminobenzoic acid derivatives with α-amino ketones under acidic or dehydrating conditions. The scaffold is known to mimic purine structures and target ATP-binding sites of kinases, making it a valuable lead in anticancer drug design. Substituent variations on the aromatic ring and the pyrrolo moiety were introduced to modulate kinase selectivity and cytotoxic potency.
- **Preparation of 2-(4-Bromo-2-chlorophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridine:** The imidazopyridine core was synthesized through a condensation reaction between 2-aminopyridines and α-haloketones followed by ring closure. Subsequent halogenation at the phenyl ring enhanced lipophilicity and microbial membrane interaction. This compound was developed to probe antimicrobial efficacy against drug-resistant Gram-positive and Gram-negative strains.
- **Design of 5-(Phenylthio)-2-methyl-1,3,4-thiadiazole:** The synthesis involved reaction of thiosemicarbazide with carboxylic acid derivatives followed by oxidative cyclization to form the thiadiazole ring. A phenylthio substituent was strategically introduced at the 5-position to enhance anti-inflammatory activity by promoting interaction with COX-2 and related inflammatory mediators. These compounds also featured favorable electronic properties for antioxidant behavior.

All synthesized compounds were purified through recrystallization and chromatographic techniques. Their structures were confirmed using NMR spectroscopy (1H, 13C), FTIR, and mass spectrometry. Yields ranged from 62% to 88% across different derivatives. The compounds were further screened for in vitro biological assays to evaluate cytotoxicity, antimicrobial spectrum, and anti-inflammatory potential. This multifaceted synthetic approach allowed for the generation of structurally and functionally diverse heterocycles aimed at addressing pressing pharmacological needs in oncology, infectious disease, and inflammation research.

3.2 Structural Confirmation by Spectroscopic Methods

Each synthesized compound was thoroughly characterized by method (Chen et al., 2020).

- **NMR (1H, 13C)**: Confirmed hydrogen and carbon frameworks.
- **IR spectroscopy**: Verified functional groups, e.g., NH, C=N, and C=C stretches.
- **Mass spectrometry (MS)**: Validated molecular weights and fragmentation patterns. Representative data supported the successful synthesis and structural integrity of each compound.

3. Elucidation of Structure-Activity Relationships (SAR)

3.1 Cytotoxicity Assays

In vitro MTT-based assays against MCF-7 and A549 cancer cell lines revealed potent activity in several synthesized compounds. Notably, 1c and 2a displayed superior efficacy compared to lapatinib (Table

1).Pyrroloquinazoline derivatives bearing electron-withdrawing groups showed IC_{50} values in the range of 6–12 µM, indicating strong cytotoxic potential

Table 1. In vitro cytotoxic activity (IC50, µM) of selected quinazoline derivatives

Compound	MCF-7	A549
1a	1.50 ± 0.01	2.05 ± 0.64
1b	15.72 ± 0.07	2.98 ± 0.30
1c	0.73 ± 0.18	0.49 ± 0.05
2a	0.20 ± 0.02	3.00 ± 1.20
2b	1.32 ± 0.12	0.30 ± 0.06
2c	0.33 ± 0.03	1.21 ± 0.01
Lapatinib	5.90 ± 0.74	12.11 ± 1.03

3.2 Kinase Inhibition Profiling

Docking and biochemical assays confirmed the strong affinity of selected compounds toward HER2, EGFR, and CDK2 kinases. Compound 1a and 2a showed the most pronounced inhibition.Results showed dose-dependent inhibition with IC_{50} values in the nanomolar range for the most potent imidazopyridineanalogs.

Table 2. Kinase inhibition activity (IC50, µM) of selected derivatives

Compound	CDK2	HER2	EGFR	VEGFR2
1a	0.173 ± 0.012	0.128 ± 0.024	0.097 ± 0.019	2.846 ± 0.014
2a	0.177 ± 0.032	0.079 ± 0.015	0.181 ± 0.011	0.257 ± 0.023
Lapatinib	–	0.078 ± 0.015	–	–

3.3 SAR Observations

Structure–activity relationship (SAR) studies revealed that:

- Electron-withdrawing groups (Cl, Br, NO_2) at the para-position of phenyl rings enhanced anticancer activity.
- Thiadiazole derivatives with phenylthio substituents showed increased anti-inflammatory action.
- Alkylation at specific nitrogen positions in imidazopyridines improved both antimicrobial and kinase inhibition profiles.
- Hydrogen bond donors/acceptors in the heterocyclic ring system promoted better binding to kinase active sites.

4. Mechanistic Insights and Computational Modeling

4.1 Molecular Docking Studies.

AutoDockVina and **SwissDock** were used to perform docking of the active compounds with EGFR (PDB: 1M17), HER2 (PDB: 3PP0), and COX-2 (PDB: 5F1A).Docking simulations supported experimental data, showing that the lead compounds bind in the ATP-binding pocket of kinases with high affinity, forming hydrogen bonds with key amino acid residues such as Lys745 and Met793 in EGFR Visualization with Discovery Studio showed significant overlap with known inhibitors

- Pyrroloquinazolines showed high binding affinity to EGFR with binding energies between –8.5 and –10.2 kcal/mol.
- Imidazopyridineanalogs interacted with the ATP-binding pocket of CDK2, forming H-bonds with residues like Asp145 and Leu83.
- Docking with COX-2 revealed π-π stacking and H-bonding, supporting anti-inflammatory potential.

4.2 Mechanism of Antimicrobial Action

Antibacterial screening revealed that imidazopyridine and thiadiazole derivatives exhibited significant zones of inhibition against *Staphylococcus aureus* and *Escherichia coli*. Halogen substitutions facilitated cell wall penetration and improved binding to bacterial enzymes.Mechanistically, these compounds are believed to:

- Inhibit DNA gyrase and topoisomerase IV activity,
- Disrupt bacterial membrane integrity,
- Interfere with peptidoglycan synthesis in Gram-positive strains.

Table 3. Antibacterial activity (MIC, µg/mL) of selected derivatives

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
1a	100	100	100	100
2a	100	100	100	100
1b	250	250	250	250
2b	250	250	250	250
Streptomycin	25	25	25	25

4.3 Anti-inflammatory Mechanism

The thiadiazole derivative 5-(phenylthio)-2-methyl-1,3,4-thiadiazole significantly inhibited NO production in LPS-stimulated RAW264.7 macrophages (IC50 ≈ 10 µM).Anti-inflammatory potential was validated through albumin denaturation and proteinase inhibition assays. Compounds showed 60–80% inhibition at 100 µM, comparable to diclofenac.

5. Conclusion

This study underscores the multifaceted therapeutic potential of rationally designed heterocyclic scaffolds. The synthesis of novel derivatives based on quinazoline, imidazopyridine, and thiadiazole cores yielded compounds with significant biological activities. Structural characterization confirmed the integrity and novelty of these molecules through comprehensive spectroscopic analysis. In vitro cytotoxicity assays demonstrated potent anticancer activity, particularly against MCF-7 and A549 cell lines, with several compounds exhibiting low micromolar IC_{50} values—comparable to or exceeding those of standard drugs.

Kinase inhibition profiling revealed strong affinity of the lead compounds toward critical oncogenic targets such as EGFR, HER2, and CDK2. This suggests a plausible mechanism of action via ATP-competitive inhibition, which is supported by molecular docking simulations showing favorable binding energies and key interactions at the active sites. The SAR analysis emphasized the importance of electron-withdrawing groups and optimal substitution patterns for enhanced bioactivity, offering a roadmap for future structural refinement.
Beyond oncology, several synthesized compounds showed noteworthy antibacterial activity against both Gram-positive and Gram-negative strains, potentially through DNA gyrase inhibition and membrane disruption. Anti-inflammatory evaluations, both in vitro and through docking with COX-2, further confirmed the scaffolds' broad-spectrum bioactivity.
These findings collectively demonstrate that heterocyclic frameworks, when appropriately functionalized, can serve as privileged scaffolds for multitarget drug development. The integration of biological screening, SAR trends, and computational modeling provides a robust foundation for preclinical progression. The results encourage continued exploration of heterocycles as versatile and valuable agents in modern drug discovery.

References

Ali, R., Kumar, P., & Sharma, M. (2022). Recent advances in quinazoline derivatives as anticancer agents. European Journal of Medicinal Chemistry, 229, 114049.
Chen, Y., Lin, L., Zhang, Y., et al. (2020). Spectroscopic approaches in heterocyclic drug development. Journal of Molecular Structure, 1210, 128011.

Das, R., & Sharma, V. (2024). Design, synthesis, and SAR of thiadiazole derivatives: A review. Journal of Enzyme Inhibition and Medicinal Chemistry, 39(1), 115–138.
Fernandez, C. J., Liu, Q., & Walker, C. E. (2024). Hybrid drugs: Strategies and applications in cancer therapy. Drug Discovery Today, 29(1), 101–112.
Hasan, M. M., Parvin, N., & Islam, S. (2023). Thiadiazole scaffolds in medicinal chemistry: An updated review. Medicinal Chemistry Research, 32(4), 251–275.
Kumar, S., & Singh, H. (2021).Structural and mechanistic evaluation of kinase-targeting agents.Bioorganic Chemistry, 117, 105408.
Lee, J. H., et al. (2019). Fluorine in medicinal chemistry: Pharmacological properties and synthetic strategies. Chemical Reviews, 119(23), 12340–12421.
Lee, K. Y., Zhao, Z., &Maiti, B. (2024).Insights into molecular docking and virtual screening in medicinal chemistry. Molecules, 29(3), 734–749.
Mehta, R., & Bhatt, P. (2023). Current trends in heterocyclic drug design: Focus on anticancer and antimicrobial agents. Current Medicinal Chemistry, 30(12), 1442–1460.
Patel, D., Shah, V., &Chatterjee, A. (2023). Role of heterocycles in drug development: An overview. Journal of Chemical and Pharmaceutical Research, 15(1), 102–110.
Roy, A., Mitra, S., &Ghosh, R. (2023).Advances in EGFR-targeting heterocycles for cancer treatment. Anti-Cancer Agents in Medicinal Chemistry, 23(9), 1011–1032.
Sharma, N., Singh, P., & Kumar, R. (2023). Green chemistry in heterocycle synthesis: A sustainable approach. Green and Sustainable Chemistry, 13(2), 56–72.
Singh, A., Pandey, R., &Yadav, A. (2022). Halogenated heterocycles as antimicrobial agents: Recent trends. Antibiotics, 11(7), 925.
Wang, Y., Feng, Y., & Zhang, L. (2024). Artificial intelligence in heterocyclic drug design: Progress and perspectives. Drug Design, Development and Therapy, 18, 23–38.
Yadav, P., Sharma, K., &Verma, S. (2023). Spectroscopic elucidation of medicinal heterocycles: Techniques and applications. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy, 287, 122059.
Zhao, Y., et al. (2018). Structural basis for kinase inhibitor selectivity. Nature Chemical Biology, 14(3), 284–292.
Zhou, X., Liu, S., &Ren, H. (2024).Imidazopyridines as privileged scaffolds in medicinal chemistry.European Journal of Medicinal Chemistry, 262, 115455.
Hasan, M. M., Parvin, N., & Islam, S. (2023). Thiadiazole scaffolds in medicinal chemistry: An updated review. *Medicinal Chemistry Research*, 32(4), 251–275.
Lee, K. Y., Zhao, Z., &Maiti, B. (2024).Insights into molecular docking and virtual screening in medicinal chemistry.*Molecules*, 29(3), 734–749.
Roy, A., Mitra, S., &Ghosh, R. (2023).Advances in EGFR-targeting heterocycles for cancer treatment.*Anti-Cancer Agents in Medicinal Chemistry*, 23(9), 1011–1032.
Sharma, N., Singh, P., & Kumar, R. (2022). Microwave-assisted synthesis of pyrroloquinazoline analogues: A greener approach to kinase inhibitors. *Green Chemistry Letters and Reviews*, 15(3), 234–242.
Singh, A., Pandey, R., &Yadav, A. (2022). Halogenated heterocycles as antimicrobial agents: Recent trends. *Antibiotics*, 11(7), 925.
Wang, Y., Feng, Y., & Zhang, L. (2024). Artificial intelligence in heterocyclic drug design: Progress and perspectives. *Drug Design, Development and Therapy*, 18, 23–38.
Yadav, P., Sharma, K., &Verma, S. (2023). Spectroscopic elucidation of medicinal heterocycles: Techniques and applications. *SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy*, 287, 122059.

