Design, Synthesis, and Evaluation of Novel Heterocyclic Compounds with Multi-Targeted Anticancer, Kinase Inhibitory, Antimicrobial and Anti-inflammatory Activities

Arunika Tiwari[1]*, Dr. Vikash Verma[2]

1 Research Scholar, Department of Chemistry, P.K. University, Shivpuri, Madhya Pradesh

dixitmayank769@gmail.com

2 Assistant Professor, Department of Chemistry, P.K. University, Shivpuri, Madhya Pradesh

Abstract: Heterocyclic compounds have long stood at the forefront of medicinal chemistry due to their remarkable pharmacological diversity and their presence in numerous therapeutic agents. This study outlines the design, synthesis, structural elucidation, and biological evaluation of a new series of heterocyclic derivatives, specifically focusing on three core scaffolds: 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. These were rationally designed for activity against cancer, microbial infections, and inflammatory conditions. Characterization was carried out using $^{1}H/^{13}C$ NMR, IR, and mass spectrometry, confirming the structural integrity of synthesized molecules. Cytotoxic testing showed that many quinazoline-based analogues inhibited kinases, including EGFR, HER2, and CDK2, and had strong inhibitory action against MCF-7 and A549 cell lines. Antimicrobial activity against both Gram-positive and Gram-negative bacteria was somewhat shown by imidazopyridine derivatives. The thiadiazole derivative showed anti-inflammatory potential by substantially inhibiting nitric oxide generation in RAW264.7 macrophages stimulated by LPS. According to structure-activity relationship (SAR) studies, the most important steps in increasing bioactivity were halogenation, sulfonamide inclusion, and electron-withdrawing substitutions. Future work includes scale-up synthesis, advanced SAR mapping, and mechanistic validation. This work underscores the utility of heterocyclic scaffolds in the development of multi-target therapeutic agents.

Keywords: Design, Synthesis, Novel, Heterocyclic Compounds, Multi-Targeted Anticancer, Kinase Inhibitory, Antimicrobial, Anti-inflammatory Activities

1. INTRODUCTION

Heterocyclic compounds form the cornerstone of contemporary medicinal chemistry, representing a substantial portion of all known pharmaceuticals. These structures are characterized by their ring systems containing at least one atom other than carbon, commonly nitrogen, oxygen, or sulfur. Their versatility in interacting with diverse biological targets, coupled with their tunable physicochemical properties, renders them indispensable in drug discovery and development (Katritzky et al., 2010). The increasing demand for safer and more effective therapeutics against multifactorial diseases such as cancer, microbial infections, and inflammatory disorders has renewed interest in the design of multifunctional heterocyclic scaffolds.

The rational design of heterocyclic molecules often begins with the identification of privileged scaffolds — structural motifs known to exhibit a broad spectrum of biological activities. Among these, quinazolines, imidazopyridines, and thiadiazoles have garnered considerable attention due to their established pharmacological relevance. Quinazoline derivatives, for instance, have demonstrated exceptional efficacy as kinase inhibitors and anticancer agents (Sharma et al., 2017). Imidazopyridines have been reported to display broad-spectrum antimicrobial properties (Singh & Sharma, 2018), while thiadiazoles are known for their anti-inflammatory and antioxidant activities (Jain & Vaidya, 2021).

Cancer, a leading cause of mortality worldwide, arises from the uncontrolled proliferation of cells driven by genetic and epigenetic aberrations. Overexpression or mutation of receptor tyrosine kinases (RTKs), such as EGFR and HER2, is a well-documented driver of tumorigenesis in several cancers, including breast and lung cancer (Zhang et al., 2016). Consequently, small molecule kinase inhibitors targeting these receptors have become a focal point of anticancer therapy. However, the emergence of drug resistance, off-target toxicity, and suboptimal selectivity necessitate the development of novel kinase-targeted agents. The incorporation of heterocycles such as pyrrolo[3,4-b]quinazolines allows for the precise modulation of physicochemical properties and binding affinities, thereby enhancing therapeutic outcomes (Anusha et al., 2016).

Likewise, the global burden of antimicrobial resistance (AMR) underscores the urgent need for innovative antibacterial agents. Conventional antibiotics are rapidly losing efficacy due to adaptive mechanisms in pathogenic microorganisms, including efflux pumps, biofilm formation, and enzymatic degradation (Ventola, 2015). The development of heterocyclic-

based antimicrobials, especially those with electron-withdrawing halogen groups, offers a promising avenue for overcoming these barriers. In instance, imidazopyridine derivatives may be effective against Gram-positive and Gram-negative bacteria by blocking DNA gyrase and topoisomerase IV (Singh & Sharma, 2018).

Inflammation is a complex immunological response to injury or infection and is implicated in numerous chronic diseases, including arthritis, neurodegenerative disorders, and metabolic syndrome. Unregulated inflammation can lead to excessive production of pro-inflammatory mediators such as nitric oxide (NO), prostaglandins, and cytokines (Aggarwal & Sung, 2009). Small molecules capable of modulating inflammatory signaling pathways, particularly those derived from heterocycles like thiadiazoles, are increasingly being explored as therapeutic agents. The thiadiazole ring offers enhanced electron delocalization, facilitating interactions with various enzymes and receptors involved in the inflammatory cascade (Xu et al., 2019).

The pursuit of multifunctional therapeutics capable of addressing cancer, infections, and inflammation concurrently is an emerging paradigm in drug discovery. Such agents not only offer broad-spectrum efficacy but also improve patient compliance and reduce the risk of polypharmacy-associated side effects. This integrative approach aligns with the principles of network pharmacology, which emphasizes multi-target modulation over the traditional “one drug, one target” model (Hopkins, 2008). In this context, designing heterocyclic compounds that can exhibit cytotoxic, kinase inhibitory, antimicrobial, and anti-inflammatory activities offers a rational and holistic therapeutic strategy.

This study presents the synthesis, characterization, and biological evaluation of a novel library of heterocyclic compounds encompassing three major scaffolds: 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. The selected scaffolds were chosen based on a robust literature survey and their proven pharmacological track records. The 3,4-dihydro-2H-pyrrolo[3,4-b]quinazoline moiety was selected for its known kinase inhibitory properties and compatibility with various substitutions that could improve solubility and target affinity. The imidazopyridine structure was selected due to its known antimicrobial efficacy, and the thiadiazole scaffold for its anti-inflammatory capabilities.

The compounds were synthesised with great care and then analysed using state-of-the-art spectroscopic methods, such as mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance (NMR). For cytotoxicity, we used the MTT assay; for anticancer, we used

kinase inhibition profiles; for antimicrobial, we used the broth microdilution technique; and for anti-inflammatory, we used nitric oxide inhibition tests. These are conventional in vitro experiments.

The novelty of this study lies not only in the unique structural variations of the synthesized compounds but also in their broad-spectrum evaluation against multiple therapeutic targets. Furthermore, structure-activity relationship (SAR) analyses were conducted to decipher the impact of different substituents on the biological performance of the compounds. This information is crucial for the future rational design of derivatives with enhanced efficacy and selectivity.

In conclusion, the integration of synthetic chemistry, spectroscopy, and biological screening in this study aims to develop lead compounds that can serve as templates for further optimization. The outcomes are expected to provide valuable insights into the design of next-generation heterocyclic therapeutics with multi-target potential.

REVIEW OF LITERATURE

The importance of heterocyclic compounds in medicinal chemistry has remained unwavering, given their integration in several FDA-approved therapeutics and their structural versatility. Among them, pyrroloquinazoline, imidazopyridine, and thiadiazole derivatives represent distinct scaffolds with diverse pharmacological applications. In this section, we present a comprehensive review of the past 15 years of literature exploring these scaffolds, especially focusing on their anticancer, kinase inhibitory, antimicrobial, and anti-inflammatory potentials.

Pyrroloquinazoline Derivatives in Anticancer and Kinase Inhibition

The pyrrolo[3,4-b]quinazoline nucleus has been a prominent structure in kinase-targeted drug development. These derivatives have shown remarkable efficacy against tyrosine kinases such as EGFR, HER2, CDK2, and VEGFR. The inclusion of pyrrole and quinazoline moieties offers significant benefits in ATP-competitive kinase inhibition (Zhang et al., 2013). Structurally, modifications at the 4- and 6-positions of the quinazoline ring with halogens, sulfonamides, or aryl ethers have demonstrated significant improvements in selectivity and potency (Sun et al., 2015; Mahapatra et al., 2020). A 2017 study by Kumar et al. reported several 4-anilino-pyrroloquinazoline derivatives with sub-micromolar IC50 values against EGFR and HER2-positive cancer cell lines. The biological results correlated strongly with

molecular docking outcomes, highlighting hydrogen bonding interactions at the ATP-binding site (Kumar et al., 2017). Other investigations have focused on hybrid scaffolds incorporating heterocycles and electron-withdrawing groups, yielding dual inhibitors for EGFR and VEGFR2 (Wang et al., 2022).Recent studies have expanded on the structure-activity relationship (SAR) of pyrroloquinazolines, with significant improvements in kinase inhibition and selectivity achieved by substituting the quinazoline core with various functional groups (Gupta et al., 2020). Additionally, the efficacy of these compounds against multidrug-resistant cancer cells has been demonstrated in several studies (Singh et al., 2019).

The ongoing exploration of these derivatives remains crucial, as resistance to first-line chemotherapies continues to challenge treatment outcomes (Gupta & Mishra, 2020).

Imidazopyridine Derivatives in Antimicrobial Therapy

Imidazo[4,5-b]pyridine scaffolds possess pronounced antimicrobial activity, particularly when functionalized with halogens and electron-withdrawing groups. This class of compounds disrupts bacterial DNA synthesis and cell wall integrity (Rahman et al., 2018). Studies show that substitutions at the 2-position with phenyl or heteroaryl groups increase lipophilicity and membrane permeability, enhancing antibacterial potency (Sinha & Ahmed, 2019). A review by Hameed et al. (2021) highlighted the potential of imidazopyridines as broad-spectrum antimicrobials.

The inclusion of bromo- or chloro-phenyl substituents has been correlated with activity against resistant strains of *S. aureus* and *E. coli*. Additional pharmacokinetic studies emphasized their stability and metabolic resistance, rendering them potential candidates for drug repurposing against neglected infections (Alamgir et al., 2020).

Recent in vitro and in vivo studies have shown their efficacy against Gram-negative bacterial strains, a major challenge in modern antimicrobial research (Patel et al., 2022). In addition, research has demonstrated the synergy of imidazopyridine derivatives with other antibiotics, offering a promising solution for overcoming antibiotic resistance (Ahmad et al., 2022). Their ability to inhibit bacterial enzymes such as DNA gyrase and topoisomerase IV has further emphasized their potential as antimicrobial agents (Sinha et al., 2020).

Thiadiazole Derivatives as Anti-inflammatory Agents

Thiadiazole scaffolds, especially 1,3,4-thiadiazoles, have shown inhibitory effects on pro-inflammatory mediators such as TNF-α, IL-6, and nitric oxide. These effects are typically mediated through iNOS and NF-κB inhibition (Jain et al., 2015).

Notably, the 5-phenylthio substitution contributes to radical scavenging and redox modulation, enhancing anti-inflammatory responses (Gupta & Mishra, 2016). Recent works by Patel et al. (2022) have demonstrated that methyl-substituted thiadiazoles with arylthio functionalities reduce LPS-induced inflammation in macrophage cell lines. Structure-activity analysis highlighted the importance of lipophilic groups in modulating cellular uptake and metabolic half-life. Furthermore, hybrid thiadiazole-based molecules incorporating NSAID fragments have yielded dual COX-2/iNOS inhibition (Sharma et al., 2019).

Thiadiazole compounds are promising options for the treatment of chronic inflammation due to their anti-inflammatory activity and their capacity to suppress the production of pro-inflammatory cytokines (Khan et al., 2021). Kumar et al. (2020) found that thiadiazoles greatly enhanced the anti-inflammatory efficiency of corticosteroid-based therapy, lending credence to the idea that combination therapy may be a more effective way to treat inflammatory illnesses.

Structure-Activity Relationship (SAR) Trends

In quinazoline derivatives, SAR studies underline the significance of electron-donating and halogen substituents in enhancing kinase binding affinity. Fluorine atoms, for instance, play a crucial role in modulating pharmacokinetics and metabolic stability (Li et al., 2016). Further studies have established that bulky substituents at the 4- and 6-positions of quinazoline improve selectivity for specific kinase targets while reducing off-target effects (Choudhury et al., 2021).

Imidazopyridine compounds benefit from bulky, halogenated aryl substitutions, enhancing antimicrobial selectivity via bacterial cell wall interactions. In thiadiazole derivatives, electron-withdrawing substituents such as NO2 and CF3 groups have consistently improved anti-inflammatory efficacy (Akhtar et al., 2020). These SAR trends emphasize the importance of molecular tailoring in the development of targeted drugs with enhanced potency and reduced side effects.

Limitations and Research Gaps

Despite their efficacy, several challenges persist. For pyrroloquinazolines, solubility and selectivity across kinase isoforms remain areas of improvement. Imidazopyridines suffer from moderate activity against Gram-negative bacteria, demanding novel delivery systems. Thiadiazoles often lack in vivo efficacy due to rapid metabolism. Thus, the present research attempts to address these limitations through rational structure-based design and biological evaluation of novel derivatives.

MATERIALS AND METHODS

Synthesis: The synthesis protocols for the heterocyclic scaffolds involved multistep synthetic procedures under controlled conditions. Quinazoline derivatives were synthesized via condensation and cyclization of anthranilic acid derivatives with aldehydes (Smith et al., 2007). Imidazopyridines were prepared through a one-pot reaction of substituted phenyl halides with 2,3-diaminopyridine (Jones et al., 2011). The thiadiazole scaffold was obtained via the reaction of thiosemicarbazides with acid chlorides in the presence of dehydrating agents (Khan et al., 2019).

Characterization Techniques: Every molecule that was synthesised underwent characterisation utilising

- **^{1}H and ^{13}C NMR Spectroscopy:** Chemical shifts were analyzed in DMSO-d_6 and $CDCl_3$ using a 400 MHz spectrometer (Ali et al., 2013).
- **FT-IR Spectroscopy:** Characteristic stretching frequencies for key functional groups such as NH, C=O, C=N, and aromatic rings were noted (Johnson & Lee, 2015).
- **Electrospray Ionization Mass Spectrometry (ESI-MS):** Mass spectrometry was used to determine molecular weights and confirm the proposed structures (Patel et al., 2016).

Biological Evaluation:

- **Cytotoxicity Assay:** MCF-7 and A549 cell lines were tested using the MTT assay, a well-established method for determining cell viability (Mosmann, 1983).
- **Kinase Inhibition:** HER2, EGFR, CDK2, and VEGFR2 assays were performed as described by Gupta et al. (2017) and Patel et al. (2018).

- **Antibacterial Activity:** MIC values were measured using the broth microdilution method (Wiegand et al., 2008).
- **Anti-inflammatory Activity:** Assessed via inhibition of NO production in LPS-induced RAW264.7 macrophages, a widely accepted method for evaluating anti-inflammatory potential (Vinegar et al., 1987).

Synthesis of Novel Heterocyclic Compounds

The synthesis of heterocyclic scaffolds was executed using stepwise synthetic routes established in medicinal chemistry literature, with necessary optimizations tailored to our target derivatives.

- **3,4-Dihydro-2H-pyrrolo[3,4-b]quinazoline derivatives** were synthesized via cyclocondensation of anthranilic acid derivatives with isatoic anhydride, followed by treatment with hydrazine and aldehydes under reflux (Zhang et al., 2014). These were further functionalized using sulfonyl chlorides or acylating agents to enhance kinase affinity (Singh et al., 2016).
- **2-(4-Bromo-2-chlorophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridine** was prepared by reacting 2-aminopyridines with α-bromoketones, followed by cyclization under acidic conditions (Alamgir et al., 2012). Halogenated phenyl moieties were introduced via nucleophilic aromatic substitution (Hameed et al., 2019).
- **5-(Phenylthio)-2-methyl-1,3,4-thiadiazole** was synthesized by reacting thiosemicarbazide with carboxylic acid derivatives and further methylation at the 2-position (Kumar et al., 2018). The arylthio substituent was introduced via a thiol exchange reaction (Sharma et al., 2014).

Characterization of the Synthesized Compounds

The synthesized compounds were thoroughly characterized to confirm their structures:

- **NMR Spectroscopy (^{1}H and ^{13}C):** Chemical shifts were analyzed in DMSO-d_6 and $CDCl_3$ using a 400 MHz spectrometer (Ali et al., 2013).
- **Infrared Spectroscopy (IR):** Characteristic stretching frequencies for key functional groups such as NH, C=O, C=N, and aromatic rings were noted (Johnson & Lee, 2015).

- **Mass Spectrometry (MS):** ESI-MS and HRMS were used to determine molecular weights and confirm the proposed structures (Patel et al., 2016).

Spectroscopic data confirmed the successful synthesis and structural integrity of all novel compounds. Representative spectra are included in the supplementary data.

Biological Assays

Anticancer and Kinase Inhibition Assays: A549 (lung cancer) and MCF-7 (breast cancer) cell lines were used in MTT tests to evaluate the anticancer potential of the compounds that were synthesised. Over the course of 48 hours, cells were exposed to the test chemicals at different concentrations. Mosmann (1983) described the spectrophotometric method used to estimate the IC_{50} values.

Kinase inhibition was evaluated using biochemical assays for EGFR, HER2, CDK2, and VEGFR2, following the methodology outlined by Gupta et al. (2017) and Patel et al. (2018). Enzyme activity was monitored via ATP consumption using luminescence-based detection (Hameed et al., 2019).

Antimicrobial Assays: Wiegand et al. (2008) introduced the broth microdilution technique as a means to evaluate minimum inhibitory concentrations (MICs) against Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, P. aeruginosa) strains of bacteria. Using streptomycin as a positive control, compounds were evaluated in triplicate.

Anti-inflammatory Assays: RAW264.7 macrophage cells were stimulated with LPS in the presence of test compounds. Nitric oxide (NO) levels were measured using the Griess assay (Vinegar et al., 1987). The IC_{50} for NO inhibition was calculated to determine anti-inflammatory potential, following the protocols set forth by Kumar et al. (2020).

RESULTS AND DISCUSSION

Novel Structures Synthesized: • 3,4-Dihydro-2H-pyrrolo[3,4-b]quinazoline: A potential scaffold for anticancer and kinase inhibition studies. • 2-(4-Bromo-2-chlorophenyl)-1,3-dihydro-2H-imidazo[4,5-b]pyridine: A novel compound with promising antimicrobial activity.

Characterization of Synthesized Compounds: The characterization of the synthesized heterocyclic compounds has been carried out using state-of-the-art spectroscopic techniques.

All compounds have been analyzed and confirmed through NMR, IR, and MS. The data were consistent with proposed structures, confirming successful synthesis. Representative spectra are included in supplementary materials.

Anticancer and Kinase Inhibition Activity: Cytotoxicity screening using MTT assay demonstrated that several derivatives exhibited potent activity against MCF-7 and A549 cancer cell lines. Notably, compounds 1c and 2a outperformed the reference drug lapatinib.

Table 1: IC_{50} values of select compounds against MCF-7 and A549 cell lines.

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

Kinase profiling confirmed strong inhibition of HER2, EGFR, and CDK2, supporting a kinase-targeted mechanism.

Table 2: Kinase inhibition profiles showing high affinity for CDK2, HER2, and EGFR.

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	–	–

SAR Observations:

Substitutions at positions 2 and 6 of the quinazoline ring affected potency. • Aryl sulfonamides and heterocycles at position 3 enhanced activity. • Fluorine substitution improved selectivity and potency.

Antimicrobial Activity Imidazopyridine derivatives were screened against S. aureus, B. subtilis, E. coli, and P. aeruginosa.

Table 3. Antibacterial activity (MIC (µg/mL) values against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*.

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

SAR Observations: • Halogenation improved membrane permeability and target binding.

Anti-inflammatory Activity The thiadiazole derivative 5-(phenylthio)-2-methyl-1,3,4-thiadiazole inhibited NO production in LPS-stimulated RAW264.7 cells ($IC_{50} \approx 10$ µM).

SAR Observations: • Phenylthio group was critical for activity. • Electron-withdrawing groups enhanced efficacy. • Methyl substitution at position 2 contributed to stability.

CONCLUSION

This study successfully synthesized and evaluated a series of novel heterocyclic derivatives incorporating quinazoline, imidazopyridine, and thiadiazole cores, aiming to explore their multi-targeted therapeutic potential. The 3,4-dihydro-2H-pyrrolo[3,4-b]quinazoline derivatives emerged as potent anticancer agents with notable kinase inhibitory activity against HER2, EGFR, and CDK2, outperforming the standard drug lapatinib in several cases (Zhang et al., 2014; Kumar et al., 2017). Structural variations, including halogenation, sulfonamide substitution, and heterocyclic integration, significantly enhanced cytotoxic activity, highlighting the importance of rational scaffold modification guided by SAR insights (Li et al., 2016; Sun et al., 2015).

Additionally, the imidazopyridine-based compounds demonstrated moderate antimicrobial effects, particularly against both Gram-positive and Gram-negative bacterial strains (Rahman et al., 2018; Sinha & Ahmed, 2019). While the activity did not surpass that of conventional

antibiotics like streptomycin, the results suggested that halogen substitutions could improve bacterial membrane permeability and enhance target binding (Alamgir et al., 2020; Hameed et al., 2021). These findings provide a foundation for further optimization to improve selectivity and potency.

The 5-(phenylthio)-2-methyl-1,3,4-thiadiazole scaffold showed promising anti-inflammatory effects, with a significant reduction in nitric oxide production in macrophage cells (Gupta & Mishra, 2016; Sharma et al., 2019). The structure-activity relationship analysis indicated that the presence of a phenylthio group and electron-withdrawing substituents were critical for biological efficacy (Jain et al., 2015). Methyl substitution at the 2-position contributed to improved metabolic stability and target interaction, marking it as a key modification for further anti-inflammatory drug development (Patel et al., 2022; Akhtar et al., 2020).

Overall, the synthesized library underscores the versatility of heterocyclic scaffolds in medicinal chemistry. The results establish a strong foundation for further SAR-driven optimization and in vivo pharmacological testing. Future directions include scaling up synthesis for selected leads, detailed mechanistic studies, and evaluating pharmacokinetic and toxicity profiles to advance these compounds toward clinical relevance (Singh et al., 2016; Kumar et al., 2020).

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