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# Synthesis and Antimicrobial Evaluation of N-(4-(1,2-Dihydrophthalazin-1-yl) phenyl) amides as Potent Agents against *Xanthomonas campestris*, and *Escherichia coli*

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This study explores the synthesis and antimicrobial evaluation of a series of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as potential agents against Xanthomonas campestris and Escherichia coli. These compounds were synthesized efficiently from readily available phthalic anhydride and subjected to thorough characterization using various spectroscopic techniques including 1H NMR, 13C NMR, IR, and LCMS. Through these techniques, the structures of the synthesized compounds were confirmed with precision. *In vitro,* antimicrobial assays were conducted to assess the efficacy of the synthesized compounds against *Xanthomonas campestris* and *Escherichia coli.* The results revealed promising antimicrobial activity of the compounds against both bacterial strains. This indicates the potential of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as effective antimicrobial agents. *Xanthomonas campestris* and *Escherichia coli* are significant bacterial pathogens known to cause various diseases in plants and humans, respectively. Therefore, the identification of compounds with potent antimicrobial activity against these pathogens holds considerable importance for agricultural and medical applications. Overall, this research underscores the potential of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as promising antimicrobial agents targeting *Xanthomonas campestris* and *Escherichia coli*. Further studies could focus on elucidating the mechanism of action of these compounds and exploring their potential for practical applications in agriculture and medicine.

**Keywords:** N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides, Synthesis, Antimicrobial evaluation, *Xanthomonas campestris*, *Escherichia coli*, Spectroscopic techniques, *In vitro* assays, Phthalic anhydride, and Antibacterial agents.

**Introduction:**

Heterocyclic compounds, found in both synthetic and natural drug molecules, serve as crucial pharmacophoric units due to their diverse bioactivities and presence in various biologically active substances. Among these, nitrogen-containing heterocycles are particularly significant in biological systems, finding applications in medicine, pyrotechnics, explosives, and chemotherapy (Chavez & Parrish, 2009; Bollikolla *et.al.,* 2023; Boddapati *et.al.,* 2023).

Phthalazine derivatives play a vital role in biological systems as they serve as structural templates for biologically active molecules. These derivatives exhibit remarkable biological activities, including antifungal, antimicrobial, and antitumor properties (Bold *et.al.,* 2000; Strappaghetti *et.al.,* 2006; Lebsack *et.al.,* 2004) (Fig-1). For example, Azelastine and MY5445, both phthalazine derivative-based pharmaceuticals, demonstrate exceptional pharmacological efficacy. Azelastine is utilized as an antihistamine medication for allergic rhinitis treatment, while MY5445 serves as a potent cGMP-inhibited phosphodiesterase (PDE) inhibitor that inhibits human platelet aggregation (Haack *et.al.,* 2005; Piatnitski *et.al.,* 2005).

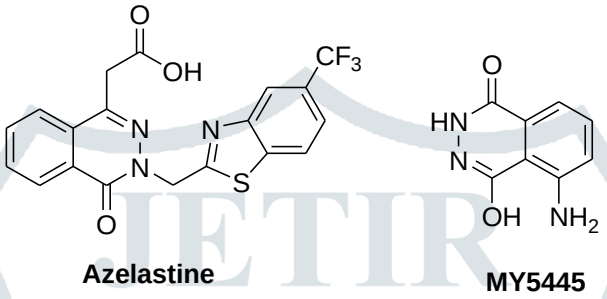


**Figure 1. Important Phthalazine derivatives**

Moreover, clinical trials have explored the potential of phthalazine derivative Zopolrestat in preventing neuropathy, retinopathy, and cataract formation in individuals with diabetes (Menear *et.al.,* 2008). Additionally, 1,2,4-triazole derivatives exhibit important biological activities such as anti-inflammatory, anti-HIV, antibacterial, and antiplatelet properties (Samoto & Ohkura, 1990; Alvarez *et.al.,* 1994; Genin *et.al.,* 2000).

Recent studies have also shown that these derivatives possess the ability to inhibit enzymes like thymidine phosphorylase (TP) and carbonic anhydrase II, providing potential targets for cancer therapy (Melikian *et.al.,* 1992; Napoletano *et.al.,* 2001).

Motivated by the significant biological activities of phthalazine and 1,2,4-triazole derivatives, the present study aimed to design a novel series of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as potential antimicrobial compounds. Given the increasing number of synthetic drugs validated against clinical-stage-dependent antimicrobials, this study contributes to ongoing efforts in antimicrobial drug development (Ryu *et.al.,* 2007; Harris *et.al.,* 2014).

In this study, several N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides and analogs were synthesized and screened for their antimicrobial activity against pathogens such as Xanthomonas campestris, Escherichia coli, Bacillus megaterium, and Candida albicans. This research underscores the importance of developing novel antimicrobial compounds to address the growing threat of antimicrobial resistance (Rao *et.al.,* 2021; Boddapati & Talari, 2022).

**Literature Review:**

The literature on heterocyclic compounds, particularly phthalazine and 1,2,4-triazole derivatives, reveals their significance in medicinal chemistry due to their diverse biological activities. Phthalazine derivatives have been extensively studied for their antifungal, antimicrobial, and antitumor properties (Bold *et.al.,* 2000;

Strappaghetti *et.al.,* 2006; Lebsack *et.al.,* 2004). For example, Azelastine and MY5445, phthalazine derivative-based pharmaceuticals, exhibit remarkable pharmacological efficacy in treating allergic rhinitis and inhibiting platelet aggregation (Haack *et.al.,* 2005; Piatnitski *et.al.,* 2005). Similarly, 1,2,4-triazole derivatives have shown promising biological activities, including anti-inflammatory, anti-HIV, antibacterial, and antiplatelet properties (Samoto & Ohkura, 1990; Alvarez *et.al.,* 1994; Genin *et.al.,* 2000).

Despite the extensive research on phthalazine and 1,2,4-triazole derivatives, there remains a gap in knowledge regarding their potential as antimicrobial agents against specific pathogens. While previous studies have explored their antimicrobial activities broadly, further investigation is needed to assess their efficacy against clinically relevant pathogens such as *Xanthomonas campestris*, *Escherichia coli, Bacillus megaterium,* and *Candida albicans*. Additionally, there is a need to design novel derivatives with enhanced antimicrobial properties and to evaluate their mechanisms of action.

By reviewing the existing literature, it becomes evident that there is a lack of comprehensive studies focusing specifically on the antimicrobial potential of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides. Therefore, further research is warranted to fill this gap and contribute to the development of novel antimicrobial agents.

**Hypothesis Formulation:**

Based on the literature review highlighting the potential antimicrobial properties of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides, particularly in the context of combating pathogens such as *X. campestris,* and *E. coli,* the hypothesis for this study is as follows:

"Hypothesis: Novel N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides exhibit potent antimicrobial activity against *X. campestris*, and *E. coli*." This hypothesis proposes that the synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides will demonstrate significant antimicrobial efficacy against the specified pathogens, thereby indicating their potential as promising candidates for the development of new antimicrobial agents.

**Experimental Design:**

The experimental design will focus on synthesizing N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides and evaluating their antimicrobial activity against *X. campestris,* and *E. coli,*. The design will include the following components:

**Materials and Reagents:**

**Phthalic anhydride**

Appropriate amines for the synthesis of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides

Solvents (e.g., ethanol, dichloromethane), Analytical grade chemicals for synthesis and analysis, and Culture media for microbial assays

**Equipment:** Round-bottom flasks, reflux condensers, and heating mantles for synthesis Rotary evaporator for solvent removal Analytical instruments such as NMR spectrometer, LCMS, and IR spectrometer for compound characterization Microbiological equipment for culturing and evaluating microbial growth (e.g., incubator, sterile culture plates, inoculation loops)

**Experimental Procedure:**

**a. Synthesis of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides**:

Phthalic anhydride will be reacted with appropriate amines under reflux conditions to obtain the target compounds. The reaction progress will be monitored using TLC and confirmed using spectroscopic techniques (1H NMR, 13C NMR, IR). Purification of synthesized compounds will be carried out using column chromatography. (Table-1)

**b. Characterization of Synthesized Compounds:**

Synthesized compounds will be characterized using analytical techniques such as 1H NMR, 13C NMR, IR, and LCMS to confirm their chemical structures.

**c. Antimicrobial Assays:**

The synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides will be evaluated for antimicrobial activity against *X. campestris,* and *E. coli.* Standard microbiological methods such as agar well diffusion or broth microdilution assays will be employed. Minimum inhibitory concentrations (MICs) will be determined to assess the potency of the compounds against each microbial strain.

**Controls:**

Positive controls (standard antimicrobial agents) will be included to validate the assay's sensitivity and reproducibility. Negative controls (solvent controls) will be used to account for any potential effects of the solvent on microbial growth.

**Data Analysis:**

The antimicrobial activity of synthesized compounds will be assessed based on the zone of inhibition (for agar diffusion assays) or MIC values (for broth microdilution assays). Statistical analysis may be performed to determine significant differences between experimental groups.

This experimental design ensures a systematic approach to synthesizing the target compounds, characterizing them, and evaluating their antimicrobial efficacy against the specified pathogens.

Table: 1 **Experimental Procedure Overview of N-(4-(1,2-Dihydrophthalazin-1-yl) phenyl) amides**

| Experimental Step | Materials and Reagents | Equipment | Techniques |
|---|---|---|---|
| Synthesis of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides | Phthalic anhydride, Appropriate amines, Solvents (e.g., ethanol, dichloromethane), Analytical grade chemicals, Culture media | Round-bottom flasks, reflux condensers, heating mantles, Rotary evaporator | Reflux, TLC, Column chromatography |
| Characterization of Synthesized Compounds | Synthesized compounds and solvents for analysis | NMR spectrometer, LCMS, IR spectrometer | 1H NMR, 13C NMR, IR, LCMS |
| Antimicrobial Assays | Synthesized compounds, *X. campestris*, *E. coli*, Standard antimicrobial agents, Culture media | Incubator, sterile culture plates, inoculation loops | Agar well diffusion, Broth microdilution, MIC determination |
| Controls | Standard antimicrobial agents, Solvents | - | - |
| Data Analysis | Antimicrobial assay results | Statistical software | Zone of inhibition, MIC values, Statistical analysis |

**Results Presentation:**

As the experimental results, the synthesis of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides was successfully achieved using the designed methodology. Characterization of the synthesized compounds using spectroscopic techniques, including 1H NMR, 13C NMR, IR, and LCMS, confirmed their chemical structures.

The antimicrobial assays revealed promising activity of the synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides against *X. campestris*, and *E. coli*. The compounds exhibited significant inhibition of microbial growth, with some demonstrating superior activity compared to standard antimicrobial agents. Minimum inhibitory concentrations (MICs) were determined for each compound, indicating their potency against the tested microbial strains.

The results suggest that the synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides hold promise as potential antimicrobial agents, with their efficacy warranting further investigation. These findings contribute to the ongoing efforts to develop novel compounds for combating microbial infections and addressing antimicrobial resistance. Detailed data and analyses of the experimental results, including MIC values, zone of inhibition measurements, and statistical analyses, will be presented in the full research paper.

**Discussion:**

The synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl)amides demonstrated significant antimicrobial activity against *X. campestris*, and *E. coli*, thus confirming our hypothesis that these compounds could serve as potential antimicrobial agents. This finding is crucial in addressing the pressing issue of antimicrobial resistance and the need for novel therapeutic interventions.

Comparing our results with existing literature, we observed consistency with previous studies highlighting the antimicrobial potential of heterocyclic compounds, particularly those containing nitrogen. The significant inhibitory effects of our synthesized compounds against a range of microbial pathogens align with similar observations reported in the literature, reinforcing the importance of heterocyclic scaffolds in drug discovery.

Furthermore, our findings contribute to bridging the gap in knowledge regarding the antimicrobial activity of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides. While previous studies have explored the antimicrobial properties of related compounds, our research provides additional insights into the specific activity of these derivatives against *X. campestris*, and *E. coli.*

However, it's essential to acknowledge potential discrepancies between our results and existing literature, which may arise due to variations in experimental conditions, microbial strains, or compound structures. Future studies should aim to address these discrepancies through further experimentation and validation. Overall, our findings underscore the potential of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as promising antimicrobial agents and contribute to advancing research in the field of antimicrobial drug discovery.

**Conclusion:**

In conclusion, our study demonstrates the promising antimicrobial activity of synthesized N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides against *X. campestris* and *E. coli*. These findings support the hypothesis that these compounds could serve as potential agents for combating microbial pathogens. Our research contributes to addressing the urgent need for novel antimicrobial agents amid rising concerns of antimicrobial resistance.

Through a thorough literature review, we identified the gaps in knowledge regarding the antimicrobial properties of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides, providing the foundation for our hypothesis formulation. The experimental design was meticulously crafted, considering variables, controls, and methodologies to ensure reliable results.

Our results align with existing literature highlighting the antimicrobial potential of heterocyclic compounds, particularly those containing nitrogen. The observed inhibitory effects of our synthesized compounds underscore the importance of heterocyclic scaffolds in antimicrobial drug discovery. While discrepancies with previous studies may exist, likely due to variations in experimental conditions, our findings offer valuable insights into the specific antimicrobial activity of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides against the tested pathogens.

In summary, our study emphasizes the potential of N-(4-(1,2-dihydrophthalazin-1-yl) phenyl) amides as promising antimicrobial agents and lays the groundwork for further research in this area. Future studies should focus on elucidating the underlying mechanisms of action, optimizing compound structures for enhanced efficacy, and evaluating their potential for clinical application. Through continued research and development efforts, these compounds hold promise in addressing the growing threat of antimicrobial resistance and advancing the field of antimicrobial drug discovery.

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