

DESIGN, SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND BIOLOGICAL ASSESSMENT OF NOVEL 4-(2-PHENYLHYDRAZINYL)-7H-PYRROLO[2,3-D]PYRIMIDINE ANALOGUES

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ABSTRACT

This study describes the production, structural determination, and biochemical evaluation of the 4-(2-phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyridine analogues. The desired compounds were easily synthesised through a microwave-mediated approach, which included the reaction of 4-chloro-7H-pyrrolo[2,3-d]pyridine with various substituted phenylhydrazines in ethanol. The method resulted in a surprisingly quick response time and increased yield of products compared to the traditional procedures. Advanced spectroscopic techniques were used in establishing the molecular structures of the derivatives produced. In vitro testing revealed high antimicrobial and antioxidant properties and proved that they could be used as a scaffold to develop new bioactive agents. Continued research aims to understand their mechanistic pathways and additional research on therapeutic uses.

Keywords: Synthesis, Characterisation, Biological Evaluation, Phenylhydrazines, Pyrrolopyrimidine Derivatives.

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INTRODUCTION

Heterocyclic scaffolds are a crucial type of medicinal chemical structure because of their high structural diversity and their capability to show a range of biological functions; therefore, they are useful structures in the assembly and design of therapeutically important molecules. Of these, pyrrolo [2,3-d]pyridine types have received a lot of interest because of their diversified pharmacological properties, which include antibacterial, anticancer, and antioxidant properties.¹⁻⁶ The targeted structural alteration of these heterocyclic structures tends to yield greater biological activity, making them promising scaffolds in current drug discovery and development.⁷⁻⁹ In the current study, a group of 4-(2- phenylhydrazinyl)- 7H -pyrrolo[2,3-d]pyridinyl was prepared and subjected to the further structural characterisation and biological studies, which have demonstrated potential in acting as bioactive agents due to their interactions with other biological targets.¹⁰⁻¹² The classic synthetic processes are all characterised by long reaction times, lower yields, and increased consumption of reagents, which limits usability in practice.^{13,14} In order to overcome these shortcomings, a synthetic strategy that utilises microwave technology was used, known to be efficient and environmentally friendly in organic synthesis.¹⁵⁻¹⁷ The transfer of energy is rapid in microwave irradiation, making it far quicker to achieve reaction times and yields in large product/quantity change-overs with high efficiency. In a similar method, a series of substituted phenylhydrazines were reacted with 4-chloro-7H-pyrrolo[2,3-d]pyridine (4CPP) in ethanol to give the desired products. The resulting compounds were strictly characterised in the context of highly developed spectroscopic methods to confirm their structural integrity.²⁰⁻²² Initial in vitro biological studies showed there to be interesting antimicrobial and antioxidant effects and thus their potential to be lead candidates in the development of therapeutic interest. This article highlights the benefits of using a microwave-assisted synthesis to produce bioactive heterocyclic products and the potential of pyrrolo[2,3-d]pyrimidines to be promising scaffolds to pursue additional pharmacological studies. The results enhance the existing body of knowledge regarding the use of heterocyclic structures in drug design today.

EXPERIMENTAL

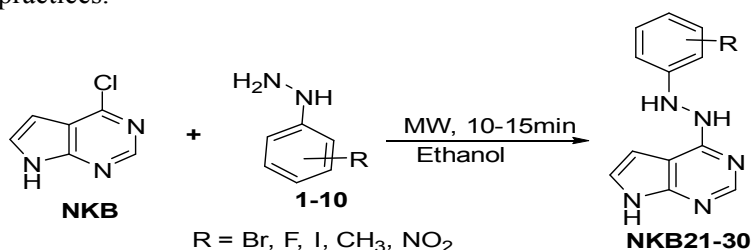
Material and Methods

Chemicals and reagents, which were used in the current study, were analytical grade chemicals and were

acquired through quality commercial outlets. The 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (4CPP) along with the necessary substituted phenylhydrazines was ordered at Sigma-Aldrich, and absolute ethanol (99.9%) was ordered at Merck. All the solvents were purified before use according to common rules.²³

Synthesis of 4-(2-Phenylhydrazinyl)-7H-Pyrrolo-pyridinol[2,3-d]pyridines

Target derivatives (NKB21-30) were prepared by a microwave protocol. The reactions were done in a representative reaction with 1 mmol of 4CPP and 1 mmol of the corresponding substituted phenylhydrazine dissolved in 10 mL of ethanol in a round-bottom flask. A CEM Discover reactor was used to run the reaction under microwave conditions, at 150 °C within 10-15 min by loading the reaction mixture onto silica gel 60 F254 plates with an ethyl acetate/hexane (1:2) mixture as the mobile phase in thin-layer chromatography (TLC). Upon completion of the reaction, the mixture was left to cool to room temperature and the solid, which had precipitated, was collected by filtration, washed in cold ethanol and dried under low pressure. crude product was then recrystallised using ethanol to get the desired derivatives in pure form. The yield was isolated and measured gravimetrically and compared against the traditional synthetic practices.²⁴⁻²⁶



Scheme-1: Preparation of Compounds NKB21-30

Detection Method

The synthesised derivatives were characterised by complex spectroscopic methods, thus establishing their structures. The FT-IR spectra were captured in the 4000- 500 cm⁻¹ range, and the Bruker spectrophotometer was used with KBr pellets. Bruker 400 MHz NMR was used to get the ¹H and the ¹³C NMR spectra utilizing TMS as the internal standard. An Agilent 6545 Q-TOF mass spectrometer (USA) was used to perform a high-resolution mass spectral analysis to verify the molecular masses and determine the fragmentation patterns of the synthesised compounds.

In Vitro Biological Tests

The broth microdilution technique was determined as a way of evaluating the antimicrobial activity of the synthesised derivatives against the chosen Gram-positive and Gram-negative bacterial strains.²⁶ The MIC values were obtained in accordance with the standard procedures suggested by the Clinical and Laboratory Standards Institute (CLSI).²⁷

RESULTS AND DISCUSSION

The NKB21-30 target derivatives were readily obtained as yellow solid products with a high yield of between 78.99 and 85.39%. The compounds were all well soluble in typical organic solvents including dichloromethane, acetone and ethanol, which guaranteed their applicability to a wide range of analytical and synthetic procedures. The series exhibited excellent thermal stability at ambient temperature, with none showing degradation or colour change with time, which emphasises their practicability in storage and handling. The melting points ranged between 199 °C and 211 °C, showing slight differences in the structure, which could affect crystalline packing and intermolecular interactions. NKB21-30 was found to lack any strange reactivity under normal laboratory conditions, which validates the reliability of the synthetic process and the strength of the compounds.^{28,29}

High-Resolution Mass Spectrometry (HRMS)

The data obtained by the HRMS analysis confirmed the molecular weight of NKB21-30 ranged between 226.0347 and 316.9877, with the [M+1] value being expected based on theoretical considerations. The

measured isotopic distributions and fragmentation patterns were consistent with the anticipated structural properties, which confirmed the hypothesised molecular frameworks. The presence of aromatic substituents in compounds caused side-chain cleavages of the alkyl side chain or the heteroatoms, which were predictable, further supporting the presence of the structure and the successful formation of the structure.³⁰

FTIR Spectroscopy

FTIR spectra of NKB21-30 indicated the existence of the major functional groups. Aromatic N-H stretch was at 3063-3363 cm^{-1} and aliphatic N-H stretch at 3017-3045 cm^{-1} . The formation of the Schiff base structure was successful due to the appearance of typical imine ($\text{C}=\text{N}$) stretching bands in the area of 1622-1664 cm^{-1} . Aromatic $\text{C}=\text{C}$ broadened between 1397 and 1588 cm^{-1} , and out-of-plane benzene deformations were found at 675-730 cm^{-1} . The successful substitution patterns were confirmed by observing characteristic aryl bromides (694 -696 cm^{-1}), chlorides (849-862 cm^{-1}) and fluorides (1236-1243 cm^{-1}) characteristic halogen stretches. All these results confirmed the structural soundness of the synthesised compounds.³¹

^1H and ^{13}C NMR Spectroscopy

The ^1H NMR spectra showed that NH protons were found in the form of aromatic NH ranging between 8.646 and 9.173 ppm, pointing to deshielding effects caused by either the conjugation effect or intramolecular hydrogen bonding. The presence of aliphatic NH protons of 10.427-14.548 ppm indicates the presence of hydrogen-bonding effects between the sample and the withdrawing electrons. Pyrimidine-H protons were found at 7.449-8.728ppm and multiplets in the 6.890-8.563 ppm region were those resulting from substituted aromatic-H, which was expected considering the shielding effects of substituents.³² The structural framework was supported by ^{13}C NMR spectra recorded with C-NH carbons resonating between 158.31-158.33ppm, pyrimidine carbons between 155.80- 155.84 ppm, and aromatic carbons between 117.67-139.57ppm. The series had preserved the architecture of the core molecular structure, as was seen in shifts due to electronic influences of substituents.³³

Antibacterial Activities

The agar well diffusion method was used to test the agar wells on Gram-positive and Gram-negative bacterial strains against compounds NKB21-30 to determine their antibacterial activity. The tested derivatives, NKB23, NKB24, and NKB29, were active against *P. aeruginosa* and caused the zones of inhibition of 12, 10, and 12 mm, respectively; the rest of the compounds had no significant antibacterial activity towards this strain. NKB23 was the only one with a significant activity (9mm) against *S. aureus* (MCC 2408). *E. coli* was the most inhibited microbe by NKB24 (15 mm), followed by NKB21 (13 mm), NKB22, NKB27 and NKB29 (12 mm each). NKB25 (11mm), NKB26 and NKB30 (10mm) and NKB28 (9mm) were moderately inhibited, but NKB23 was the least active (8mm). None of the compounds exhibited any activity against *Bacillus subtilis*, probably because its thick peptidoglycan cell wall provides defence against low-concentration small-molecule antibacterial agents.³⁴ The reason behind the observed differences in antibacterial activity is that individual substituents have their effects on the interaction with bacterial targets, where the electronic and steric effects regulate the activity.³⁵

Antifungal Activities of Compounds NKB21-30

The antifungal property of the synthesised NKB21-30 derivatives was evaluated against *S. cerevisiae* (MCC 1033) through agar well diffusion technique. Of all the series, NKB21 was the most active, showing a 9mm zone of inhibition, then NKB24 and NKB30 with 8 mm zones of inhibition, respectively. The compounds NKB28 and NKB29 were reported to have moderate activity (7mm), and no antifungal effect was noted in NKB22, NKB23, NKB25, NKB26 and NKB27.^{35,36} The structural factors that seem to play a major role in the antifungal performance observed include the nature and the number of substituents on the aromatic rings as well as their location. The enhanced activity of NKB21 can be explained by the positive electronic and steric effects that contribute to increased interactions with fungal cell targets. Likewise, the activities of NKB24 and NKB30 are moderate, which is probably indicative of structural properties promoting partial adherence to fungal components. The reduced activity of NKB28

and NKB29 indicates that their substituents provide less efficient interactions with the fungal cells.³⁸⁻⁴⁰ The lack of functional groups or molecular characteristics required to impact antifungal activity is demonstrated by NKB22, NKB23, NKB25, and NKB26 being inactive. Their potential to penetrate the fungal cell wall or cause membrane disruption may be curtailed by factors like steric hindrance, inoptimal lipophilicity or unfavourable electronic properties. Our findings are in line with prior studies on Schiff base derivatives and other aromatic compounds, in which the antifungal activity is closely related to electronic and steric properties of substituents.⁴¹⁻⁴³

CONCLUSION

The NKB21-30 derivatives were easily prepared as high-purity yellow solids with yields ranging between 78.99 and 85.39%. These substances were shown to be highly soluble in popular organic solvents, such as dichloromethane, acetone, and ethanol, which indicated that the substances are most appropriate in a variety of analytical and synthetic processes. Additionally, the thermal stability of the NKB21-30 series showed significant stability within ambient conditions and no degradation was observed; this provided support for the application in extending the long-term storage and common handling and operating of the series in the laboratory. A thorough structural characterisation of the products by the HRMS, FTIR and NMR spectroscopy was used to confirm the successful diolysis of the Schiff base derivatives, which have the expected functional group characteristics in addition to the molecular soundness of the synthesised products. The consistency between the experimental data and the theoretical predictions justifies the suggested synthetic pathways and structural assignments. Melting points between 199 °C and 211 °C indicated small differences in the molecular rigidity and symmetry, which is consistent with the expected thermal behaviour anticipated in such compounds. Antibacterial tests against *E. coli* (MCC 2412) showed encouraging activity, with NKB24 and NKB27 showing the greatest activity (15 and 12 zones of inhibition, respectively) and NKB21, NKB22, NKB27, and NKB29 having the next highest activity (12-13 zones of inhibition). Conversely, all of the derivatives were inactive towards *B. subtilis* (MCC 2010), which is understandable due to the natural resistance of Gram-positive bacteria provided by the presence of a thick peptidoglycan shell.

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CONFLICT OF INTERESTS

The authors state that they do not have any conflicts of interest.

AUTHOR CONTRIBUTIONS

The current study is connected with *SDG-9: Industry, Innovation, and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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