Original Research Article

Synthesis and Antimicrobial Profiling of a Pyrazole-Based Schiff Base Incorporating 2,4-Dihydroxybenzaldehyde

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ABSTRACT

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| In this study, a Schiff base (DHB-Pz) was synthesized via a condensation reaction between 5-amino-3-methyl-1-phenylpyrazole and 2,4-dihydroxybenzaldehyde. The compound was characterized using elemental analysis, FTIR, UV-Vis spectroscopy, and conductivity measurements. The FTIR spectrum confirmed the formation of the azomethine linkage (C=N) through a distinct stretching band, while UV-Vis absorption at 412 nm indicated extended conjugation. The Schiff base was screened for antimicrobial activity against a panel of Gram-positive (Bacillus subtilis, Serratia sp.) and Gram-negative (Klebsiella sp., Proteus sp.) bacteria, as well as fungi (Candida albicans and Aspergillus niger) using the agar well diffusion method. Minimum inhibitory concentrations (MICs) were determined using serial dilution techniques. The compound exhibited broad-spectrum antimicrobial activity with MIC values as low as 0.312 mg/mL for Aspergillus niger, and comparable potency to reference drugs (Gentamycin and Nystatin). The promising activity may be attributed to the presence of hydroxyl groups, the pyrazole moiety, and the conjugated system, which enhance membrane interaction and disruption. These findings underscore the potential of DHB-Pz as a scaffold for future antimicrobial agent development |

*Keywords: Schiff base, Pyrazole derivatives, antimicrobial activities, minimum inhibitory concentration (MIC), 2,4-Dihydroxybenzaldehyde*

1. INTRODUCTION

The alarming rise in antimicrobial resistance has become a major threat to global health, necessitating the discovery of novel bioactive compounds with potent antimicrobial properties. Schiff bases, formed via condensation of primary amines and aldehydes or ketones, have been extensively studied for their biological activities, including antibacterial, antifungal, and anticancer effects (Singh, et al., 2006; Rollas and Küçükgüzel, 2007). The imine (–C=N–) functional group is central to the biological activity of Schiff bases, where it facilitates coordination with biological macromolecules and promotes interactions with microbial enzymes and cell membranes. Schiff bases are well-documented for their antimicrobial properties, primarily due to their capacity to form stable chelates with metal ions and disrupt essential cellular processes in pathogens (Patel and Pandeya, 2011; Chohan and Supuran, 2001). Structural variations among Schiff bases, including the incorporation of heterocyclic rings and aromatic moieties, have been shown to significantly influence antibacterial and antifungal activity (Joseyphus and Sivasankaran, 2008; Jarrahpour et al., 2007). For example, Schiff bases derived from pyrazolone backbones have demonstrated not only antimicrobial efficacy but also notable cytotoxic and antioxidant profiles (Aly et al., 2010; Sashidhara et al., 2008).

Beyond microbial inhibition, Schiff bases have also found application in herbicidal and oxidative stress-related studies due to their diverse electronic characteristics (Samadhiya and Halve, 2001; Neochoritis et al., 2011). Functional group modifications—such as the inclusion of long alkyl chains or isoniazid derivatives—can enhance membrane permeability, bioavailability, and overall antimicrobial efficacy (Shaker et al., 2011; Yong et al., 2016). More advanced Schiff base frameworks, especially those incorporating chiral or extended aromatic systems, have been explored for their anti-inflammatory and anticancer potential, reinforcing the relevance of this class of compounds in multidisciplinary drug design (Zhang et al., 2009; Zhou et al., 2010).

Among the various structural motifs explored, pyrazole-based Schiff bases stand out due to their electron-rich aromatic framework, π-conjugation, and capacity for hydrogen bonding, all of which enhance their interaction with biological targets such as enzymes and nucleic acids (Bharti et al., 2012). These heterocyclic scaffolds are widely recognized in pharmaceutical research for their diverse pharmacological profiles, including anti-inflammatory, anticancer, and antimicrobial properties (Kumar and Chopra, 2020; Kaur and Pathania, 2021). When conjugated with phenolic aldehydes, the resulting Schiff bases often display improved lipophilicity and bioavailability. Hydroxyl-substituted aromatic aldehydes, in particular, contribute significantly to antimicrobial potency by promoting hydrogen bonding and modulating lipophilicity, which facilitates cellular uptake (Nassar et al., 2020).

The combination of hydroxyl groups and pyrazole functionalities in a Schiff base is known to enhance antimicrobial performance through mechanisms such as metal chelation, membrane disruption, and enzyme inhibition (Mohamed and Omar, 2005). For instance, Bharti et al. (2012) synthesized a series of molybdenum(0) pyrazolone-based Schiff base complexes and reported that the biological activity was strongly influenced by the presence of hydroxyl and aromatic moieties, which enhanced microbial enzyme interaction. Similarly, Nassar et al. (2020) reported Schiff bases derived from hydroxybenzaldehydes and pyrazole analogues exhibit significant antibacterial and antifungal activity, underscoring the role of these substituents in enhancing bioactivity.

Despite the wide array of Schiff base derivatives documented in literature, the direct investigation of antimicrobial activity from a Schiff base combining 5-amino-3-methyl-1-phenylpyrazole with 2,4-dihydroxybenzaldehyde remains limited. This study, therefore, aims to synthesize and characterize such a Schiff base—designated DHB-Pz—and evaluate its antimicrobial efficacy against selected Gram-positive and Gram-negative bacterial strains, as well as fungal species. The work contributes a new structural variant of bioactive Schiff base to the expanding field of antimicrobial drug discovery.

2. material and methods

**2.1 Materials and Reagents**

All chemicals used in this study were of analytical grade and used without further purification. 2,4-Dihydroxybenzaldehyde and 5-amino-3-methyl-1-phenylpyrazole were purchased from Sigma-Aldrich. Solvents such as ethanol, methanol, acetone, and dimethyl sulfoxide (DMSO) were obtained from Merck. Gentamycin and nystatin were used as standard control drugs for antibacterial and antifungal studies, respectively. The FTIR spectra were recorded using a PerkinElmer Spectrum Two FTIR Spectrometer using the KBr pellet method. UV-Visible spectra were obtained on a Shimadzu UV-1800 spectrophotometer using methanol as the solvent. Elemental analysis (C, H, N) was performed using a PerkinElmer 2400 Series II CHNS/O analyzer. Conductivity measurement carried out using DDS-307 conductivity meter.

**2.2 Synthesis of Dihydroxybenzaldehyde–Pyrazole Schiff Base (DHB-Pz)**

The Schiff base, DHB-Pz was synthesized via a condensation reaction. Equimolar amounts (10 mmol) of 2,4-dihydroxybenzaldehyde and 5-amino-3-methyl-1-phenylpyrazole were dissolved in ethanol (30 mL) and refluxed for 4 hours with continuous stirring. The resulting yellow precipitate was filtered, washed with cold ethanol/acetone solution and dried in a desiccator. The crude product was recrystallized from ethanol to obtain a pure Schiff base compound.Yield: 61.4%; mp: 183.3 °C; Anal (calc.): C 69.32 (69.61), H 5.11 (5.15), N 14.12 (14.33).

**2.3 Antimicrobial Activity**

**2.3.1 Microbial Strains**

The antimicrobial activity of the synthesized DHB-Pz was tested against Gram-positive bacteria: *Bacillus subtilis*, *Serratia sp*.; Gram-negative bacteria: *Klebsiella sp*., *Proteus sp*.; Fungi: *Aspergillus niger* (mold), *Candida albicans* (yeast). All microbial strains were obtained from Microbiology Laboratories of University of Uyo, Nigeria.

**2.3.2 Antimicrobial screening**

The antimicrobial activity of the Schiff base was tested using the agar well diffusion method. A stock solution of 20 mg/mL was prepared in DMSO and serially diluted to obtain concentrations of 10, 5, 2.5, and 1.25 mg/mL (Bashir and Siraj, 2021; Shettima et al., 2024). Sterile Mueller-Hinton agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) were used. Wells of 6 mm diameter were bored into the agar and filled with 100 µL of each test solution. Plates were incubated at 37°C for 24 hours (bacteria) and 48 hours at 28°C (fungi). The zones of inhibition (ZI) were measured in millimeters. Gentamycin (20 mg/mL) and Nystatin (20 mg/mL) served as positive controls for bacteria and fungi, respectively. DMSO was used as the negative control.

**2.3.3 Determination of Minimum Inhibitory Concentration (MIC)**

The MIC was determined using the agar incorporation method combined with streak inoculation. The lowest concentration at which the compound showed activity was further serially diluted in DMSO to yield concentrations of 2.5, 1.25, 0.625, and 0.312 mg/mL. One milliliter (1 mL) of each diluted solution was added to 19 mL of sterile molten agar maintained at 45°C, mixed thoroughly, poured into sterile Petri dishes, and left to set. The plates were then allowed to solidify completely at room temperature under aseptic conditions. Sensitive test organisms were inoculated using the streaking method. Plates were incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi. and monitored for visible growth. The MIC was recorded as the lowest concentration of the Schiff base that inhibited microbial growth.

3. results and discussion

**3.1 Synthesis and Physical properties**

The Schiff base DHB-Pz (3) was obtained as a yellow crystalline solid via the condensation reaction of 2,4-dihydroxybenzaldehyde (1) with 5-amino-3-methyl-1-phenylpyrazole (2) in ethanol (Scheme 1). The product was formed in high yield and exhibited moderate solubility in methanol and DMSO, sparing solubility in ethanol and acetone, and was insoluble in water. The observed solubility trend is consistent with the moderate polarity of DHB-Pz, attributed to the presence of both hydrophobic (aromatic rings) and hydrophilic (hydroxyl and azomethine groups) moieties. Conductivity measurement of the compound in DMSO showed a value of 6.87 µS/cm, indicating its non-electrolytic nature and confirming that it does not undergo significant ionization in solution. This property supports the existence of the compound as a neutral molecular species.

**Pic 1**. **Preparation of the DHB-Pz Schiff base**,



**3.2 Elemental Analysis**

Elemental analysis data revealed close agreement between the calculated and experimental percentages: C (69.61% calc., 67.32% found), H (5.15% calc., 5.11% found), and N (14.33% calc., 14.12% found). These values are consistent with the proposed molecular formula, C₁₇H₁₅N₃O₂, confirming the purity and composition of the synthesized Schiff base.

**3.3 FTIR Spectroscopy**

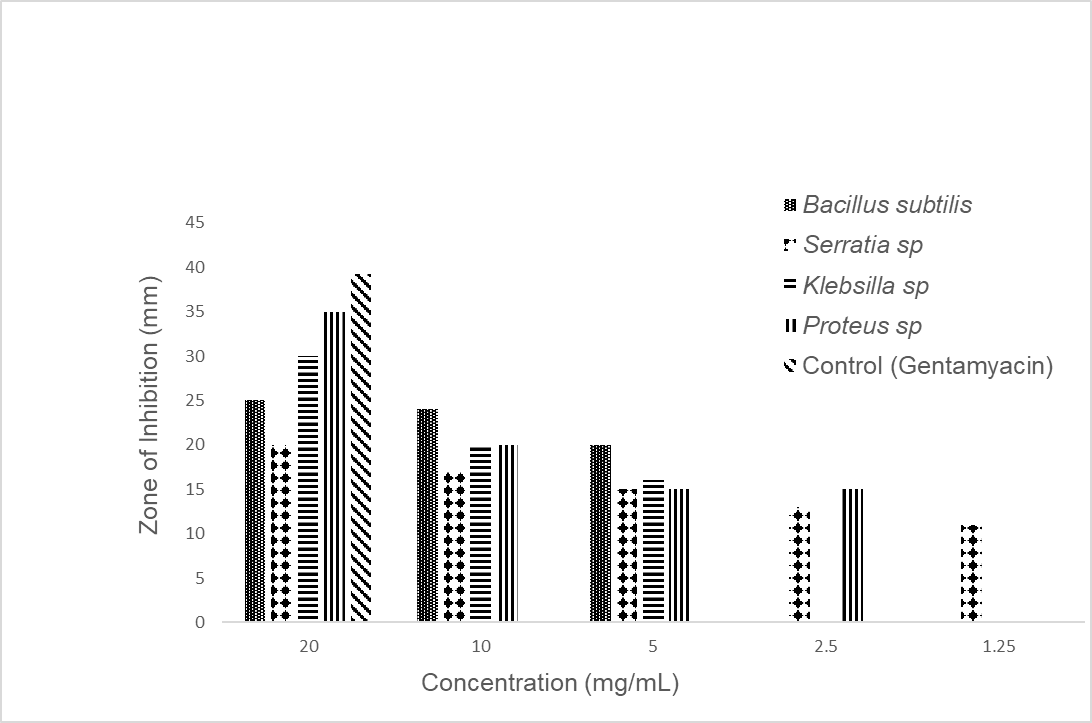
The FTIR spectrum of DHB-Pz exhibited a characteristic sharp band at 1559 cm⁻¹, attributed to the C=N stretching vibration of the azomethine linkage, confirming successful Schiff base formation. Additional absorption bands at 2877 and 2832 cm⁻¹ correspond to aliphatic C–H stretching, especially from the methyl group on the pyrazole ring. Notably, the broad hydroxyl stretching band expected near 3350 - 3500 cm⁻¹ was diminished, and a weaker broad absorption was observed around 3652 cm⁻¹. This reduction is likely due to intramolecular hydrogen bonding between the hydroxyl and imine nitrogen atoms. The disappearance of the N–H stretching vibration of the primary amine expected around 3300–3400 cm⁻¹ and that of C=O stretching vibration of the aldehyde (around 1680–1720 cm⁻¹) further supports the formation of the Schiff base.

**3.4 UV-Visible Spectroscopy**

The UV-Vis spectrum of DHB-Pz in methanol exhibited absorption bands at 209 nm and 211 nm, which are characteristic of π→π\* transitions. These transitions are typical of aromatic systems and are attributed to the π-electron systems of the benzene rings and the pyrazole moiety (Silverstein et al., 2005). The proximity of the two bands suggests overlapping absorptions from the conjugated aromatic fragments present in the Schiff base. A more intense band at 412 nm was observed and assigned to n→π\* transitions, likely involving lone pair electrons on the imine nitrogen and possibly the pyrazole nitrogen. The imine nitrogen is strongly conjugated with the benzene ring system (from 2,4-dihydroxybenzaldehyde), and its lone pair is involved in extended delocalization (Mounika et al., 2010). The pyrazole nitrogen(s) may be partly conjugated with the ring or more localized. The pyrazole nitrogen likely plays a secondary, supportive role by enhancing conjugation and lowering the energy gap, helping the n→π\* transition shift even further into the visible region. This bathochromic shift supports the Schiff base’s chromophoric nature and is consistent with the observed yellow color of the compound.

**3.5 Antimicrobial Activity**

The Schiff base DHB-Pz displayed notable antimicrobial activity against both Gram-positive and Gram-negative bacteria (Fig. 1), as well as fungi (Fig 2). Among bacteria, the largest zone of inhibition was recorded for *Proteus sp*. (35 mm at 20 mg/mL), followed by *Klebsiella sp*. (30 mm), *Bacillus subtilis* (30 mm at 5 mg/mL), and *Serratia sp*. (20 mm). Fungal inhibition was also promising, with *Candida albicans* showing a zone of 25 mm and *Aspergillus niger* showing 20 mm at 20 mg/mL. The activity was concentration-dependent. When compared to reference drugs - Gentamycin (bacteria) and Nystatin (fungi) - DHB-Pz showed comparable zones of inhibition, especially in the case of *Candida albicans*, where the Schiff base (25 mm) outperformed Nystatin (19 mm). The observed activity may be attributed to the compound’s ability to permeate and disrupt microbial membranes, enhanced by its planar structure and presence of hydroxyl and pyrazole moieties. While the presence of the hydroxyl groups and the conjugated pyrazole ring may facilitate interactions with the bacterial cell membrane, enhancing permeability and leading to leakage of essential cellular components, the planar structure may facilitate easier interaction with the lipid bilayer, a crucial factor in membrane disruption (Aragón-Muriel et al., 2021).

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**Fig. 1. Antimicrobial activity of DHB-Pz against selected bacterial strains**

**Fig. 2. Antimicrobial activity of DHB-Pz against selected fungal strains**

**3.6 Minimum inhibitory concentration (MIC)**

MIC is a critical parameter that determines the lowest concentration of an antimicrobial agent required to completely inhibit the visible growth of a microorganism. MIC determination (Fig. 3) revealed that *Aspergillus niger* had the lowest MIC value of 0.312 mg/mL, followed by *Serratia sp*., *Klebsiella sp*., *Proteus sp*., and *Candida albicans* (all at 0.625 mg/mL). *Bacillus subtilis* showed a higher MIC value of 5 mg/mL. These findings highlight that the Schiff base was more effective against Gram-negative bacteria and fungi than against Gram-positive species. When compared to literature reports of some antimicrobial agents (Parvekar et al., 2020; Qaralleh et al., 2020), the MIC values observed here are within a comparable range, validating the compound’s potency and therapeutic relevance. These results suggest that DHB-Pz can serve as a promising scaffold for further development of antimicrobial agents.

**Fig. 3. Minimum inhibitory concentration of DHB-Pz on test microorganisms**

4. Conclusion

A Schiff base (DHB-Pz) was successfully synthesized via condensation of 5-amino-3-methyl-1-phenylpyrazole with 2,4-dihydroxybenzaldehyde. The compound was characterized using elemental analysis, FTIR, UV-Vis spectroscopy, and conductivity measurements, all of which supported the proposed structure. The Schiff base exhibited good solubility in polar organic solvents and was found to be non-electrolyte in nature.

Biological screening revealed broad-spectrum antimicrobial activity against both bacterial and fungal strains. Notably, the DHB-Pz demonstrated considerable inhibitory effects against *Proteus sp*., *Klebsiella sp*., and *Candida albicans*, with MIC values ranging from 0.312 to 5 mg/mL. DHB-Pz's activity was comparable to standard antibiotics, reinforcing its therapeutic potential.

The promising antimicrobial performance of DHB-Pz may be attributed to its conjugated planar structure, phenolic hydroxyl groups, and pyrazole moiety, which may enhance membrane interactions. These findings indicate that DHB-Pz is a viable candidate for further development as an antimicrobial agent.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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