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Original Article

Synthesis, Pharmacological, and Hematological Evaluation of Cinnamaldehyde-Derived Schiff Base

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ABSTRACT

Background: Cinnamaldehyde (*trans*-cinnamaldehyde) and *para*-aminophenol, (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol were used in the synthesis of Schiff base. This study aimed to synthesize and quantify derivatives of *para*-aminophenol and Cinnamaldehyde Schiff bases derivative, characterize and determine the toxicity and pharmacological properties, as well as potential binding affinity on specific cellular enzymes and proteins.

Methods: The compound was synthesized via microwave-assisted condensation, achieving a high yield of 95.4%, and characterized using physicochemical analysis, thin-layer chromatography (Rf = 0.69 ± 0.08 in methanol), ^{1}H NMR (nuclear magnetic resonance), ^{13}C NMR, a high-resolution mass spectrometer, and UV-visible spectrophotometry (λmax = 260 nm). Biological evaluations included antimicrobial, acute toxicity (LD_{50}), analgesic, and hematological profiling such as hemoglobin concentration, packed cell volume, platelet count, and differential leukocyte distribution.

Results: The compound demonstrated moderate antimicrobial activity, with zones of inhibition against *Escherichia coli* (13 mm at 62.5 µg/mL) and *Candida albicans* (13 mm at 500 µg/mL). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were found to be 250 µg/mL for *E. coli* and 62.5 µg/mL (MIC) for *C. albicans*. Analgesic evaluation revealed that the test compound significantly increased reaction time in mice, particularly at 60- and 90-minute post-administration, with effects comparable to pethidine (at a dose of 12 mg/kg). ANOVA confirmed significance (P = 0.0002), while no significant difference was found between the test compound and the standard at peak effect (P = 0.80). LD_{50} of 1265 mg/kg suggests a moderate toxicity profile. Hematological analysis showed dose-dependent changes in white blood cell and red blood cell counts. *In silico* docking studies demonstrated a strong binding affinity to key antimicrobial and analgesic targets, including Cyclooxygenase-2 (COX-2) (−7.52 kcal/mol) and 5FSA (−7.65 kcal/mol), which supports the *in vivo* results.

Conclusions: These findings suggest that the synthesized Schiff base compound has promising potential as a dual-action antimicrobial and analgesic agent, with a relatively safe hematological profile and good molecular interactions at target sites.

Key words: Schiff base, cinnamaldehyde, *para*-aminophenol, analgesic, antimicrobial, hematology

INTRODUCTION

The reaction of *para*-aminophenol (PAP) with aromatic aldehyde derivatives, including benzaldehydes and cinnamaldehyde, has been reported to produce various imines (Schiff bases), which are useful in multiple analytical and medicinal applications. [1] PAP is synthesized from phenol through nitration with nitric acid, followed by reduction with iron. Alternatively, it can be produced by the partial hydrogenation of nitrobenzene to form phenylhydroxylamine, which then rearranges primarily into PAP. [2] PAP is an aminated phenolic compound with the amino group positioned para to the phenolic –OH group, that functions as both a metabolite and an allergen. [3]

The estimated oral lethal dose of PAP for humans ranges from 50 to 500 mg/kg, equivalent to between 1 teaspoon and 1 ounce for a 70 kg individual. In experiments using hepatocytes from male Sprague-Dawley rats, PAP was metabolized into two major metabolites, PAP-glutathione (GSH) conjugates and PAP-N-acetylcysteine conjugates, along with several minor metabolites. Pre-treatment of hepatocytes with 1-aminobenzotriazole, a Cytochrome P450 (CYP450) enzyme inhibitor, is not known to alter the PAP metabolic pattern. These findings suggest that PAP is primarily metabolized into PAP-GSH conjugates and PAP-N-acetylcysteine conjugates in hepatocytes, with these metabolites present in sufficient quantities, leading to nephrotoxicity. [4] One of the primary effects of PAP exposure is the formation of methemoglobin, characterized by abnormally high levels of methemoglobin (MetHb), where the iron in hemoglobin is oxidized from ferrous (Fe^{2+}) to ferric (Fe^{3+}). Methemoglobin levels above 1% are considered abnormal **(Figure 1)**. The oxidation of hemoglobin to methemoglobin disrupts its oxygen-carrying capacity, potentially leading to chemical asphyxia when it exceeds 60%. It is hypothesized that PAP forms covalent bonds with the reactive –SH groups of hemoglobin, transferring electrons to oxygen and thereby generating methemoglobin. [5] PAP is a potent reducing agent, serving as a corrosion inhibitor in paints and as an anti-corrosion lubricant in two-cycle engine fuels. [6] It also functions as a chemical intermediate in the production of various medicinal compounds, including paracetamol. [7,8]

Cinnamaldehyde is an organic compound that occurs naturally as the trans-(*E*) isomer, responsible for the characteristic flavor and aroma of cinnamon, and is synthesized via the shikimate pathway. Found predominantly in the bark of cinnamon trees, it constitutes about 90% of cinnamon bark essential oil. [9]

Cinnamaldehyde can be synthesized in the laboratory through various methods, but it is most economically obtained by steam-distilling cinnamon bark oil. It can also be prepared from cinnamyl alcohol or via the aldol condensation of benzaldehyde and acetaldehyde. Biosynthetically, it begins with the deamination of L-phenylalanine to cinnamic acid, catalyzed by phenylalanine-ammonia-lyase (PAL). This is followed by the conversion of cinnamic acid to cinnamoyl-CoA by 4-coumarate-CoA ligase (4CL), and finally, cinnamoyl-CoA is reduced by nicotinamide adenine dinucleotide phosphate (NADPH) to form cinnamaldehyde. [10–12] Cinnamaldehyde is widely used in flavoring chewing gum, ice cream, candy, and beverages at concentrations ranging from 9 to 4900 ppm. It is also incorporated into perfumes for its natural, sweet, and fruity scents, contributing to aromas like almonds, apricots, and butterscotch. [13] Additionally, cinnamaldehyde has been tested as an effective insecticide against mosquito larvae, with a concentration of 29 ppm killing half of Aedes aegypti larvae within 24 hours. [14] It also acts as a potent fumigant and repellent for adult mosquitoes. [15]

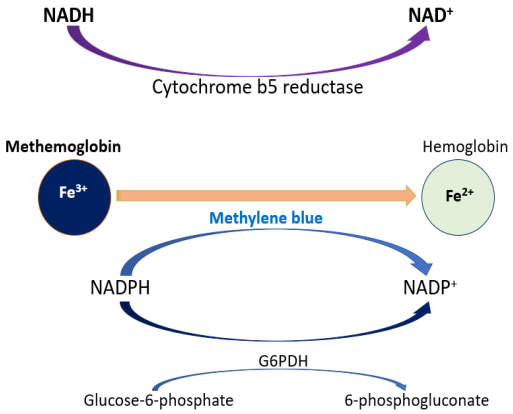

Figure 1: Mechanism of methemoglobin formation.

The synthesis of Schiff bases is commonly carried out by refluxing a mixture of equimolar primary amines and aldehydes in non-aqueous organic solvents for 8 to 12 hours. This reaction is typically acid-catalyzed and may require an azeotroping agent or the removal of the water produced to drive the reaction forward. [16–18] Since the reaction is highly reversible, removing the water generated is crucial for achieving the highest yield of Schiff bases. [19,20] Microwave-assisted organic reactions have emerged as a modern tool in organic compound synthesis, offering notable advantages such as significantly faster reaction rates, reduced reaction times, improved yields, better product quality, and simpler procedures compared to traditional methods. [16] This technique is considered an environmentally friendly approach under *Green Chemistry* due to its sustainable properties. The use of microwaves in organic synthesis has been proven to be efficient, safe, and environmentally gentle, with shorter reaction times. [21,22] The study aimed to synthesize and quantify derivatives of PAP and Cinnamaldehyde Schiff bases, characterize and determine the toxicity and pharmacological properties, as well as potential binding affinity on specific cellular enzymes and proteins.

MATERIALS AND METHODS

Chemical Reagents and Equipment

Cinnamaldehyde (*2E*-3-phenylprop-2-enal) (Loba Chemie Ltd, India), PAP (Macklin®), ethanol (Scharlau, Spain), methanol (Loba Chemie Ltd, India), diethyl ether, ethyl acetate (Guangdong Sci-Tech), ammonia solution, nitric acid (HNO_3), glacial acetic acid (Riedel-de Haen), *n*-hexane (Merck, Darmstadt, Germany), *n*-butanol, dimethyl sulfoxide (DMSO; Loba Chemie, India), petroleum spirit, sodium borohydride ($NaBH_4$), sulfuric acid, ferric chloride, ethyl acetate, acetic acid, acetic anhydride, dichloromethane (Loba Chemie, India), resorcinol (Guangdang Co. Ltd, China), chloroform, diethyl ether, paracetamol powder, and fluconazole. Colorimeter, reflux condenser, petri dish, Gallenkamp melting

point apparatus (England), thermometer, hot water bath, analytical weighing balance, spatula, magnetic stirrer, pH meter (Hanna Instruments Inc. USA–ISTRPHEPR1), thin-layer chromatographic (TLC) plates (Merck, Sigma-Aldrich, Canada), UV/visible spectrophotometer, nuclear magnetic resonance (NMR; BRUKER AVANCE III), high-resolution mass spectrometer (HRMS; Agilent).

Clinical Isolates and Laboratory Animals

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans* were used in the antimicrobial screening. All these organisms were collected from the laboratory stock and stored in the incubator. Swiss albino mice, of both sexes, weighing 16.9 to 25.3 g, were used for acute toxicity (LD_{50}), analgesic activity, and hematological screening.

Synthesis of (Z)-4-((4-(Dimethylamino)Benzylidene)-Amino)-Phenol

A weighed quantity of PAP (3.0 g, 0.1 M) and cinnamaldehyde (2*E*-3-phenylprop-2-enal) (3.3 g, 0.1 M) were dissolved in 25 mL of methanol. A few drops (2–3) of glacial acetic acid were added to the mixture in a Pyrex beaker and irradiated in a microwave at a power of 20% intensity (140 W) for about 5 minutes. The resultant solution was allowed to stand at room temperature and poured into cold water. The solid separated was filtered and recrystallized from methanol. [23] TLC was used to ascertain the completion of the reaction. This gave sample (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol. Melting point and other physicochemical parameters were determined and recorded, respectively.

Physicochemical and Spectrophotometric Analysis

The percentage (%) yield was calculated using the initial mass of reactants and the final mass of the product. Some quantity of the products was transferred into a test tube, and 5 mL of water was used to dissolve the sample. This was repeated with DMSO, ethanol, and methanol to test solvent solubility. 100 mg of paracetamol and the synthesized sample were dissolved in 100 mL of methanol, making a 1 mg/mL (1000 µg/mL) stock solution. Different concentrations, ranging from 2, 4, 8, 16, and 32 µg/mL, respectively, were serially prepared from the stock solution. These concentrations were used to scan between 220 and 900 wavelengths in a UV spectrophotometer, and the maximum absorbance (λmax) was recorded. The proton (^{1}HNMR) and carbon-13 (^{13}CNMR) parameters of the samples were characterized using NMR, AscendTM 400 Bruker. NMR was collected from a BRUKER AVANCE III 400/500/600 frequency (AscendTM). HRMS–electrospray ionization (ESI) was used to determine the splitting pattern of the synthesized compounds. HRMS was collected from an Agilent 1290-6545 UHPLC-QTOF.

Antimicrobial Screening

About 0.1 g (100 mg) of (Z)-4-((4-(dimethylamino) benzylidene)-amino)-phenol was weighed and dissolved in 10 mL of DMSO. Ninety milliliters of distilled water were added to the solution to form a stock solution of 1000 µg/mL. Five concentrations (500, 250, 125, and 62.5 µg/mL) were prepared. A pure culture of each clinical isolate (*E. coli, S. aureus,* and *C. albicans*) was standardized by inoculating a loopful of the organism into a sterile bottle containing 5 mL of normal saline, and the turbidity was adjusted and compared to the 0.5 McFarland Standard. A standardized overnight bacterial culture was spread twice over sterilized Mueller-Hinton Agar plates for even distribution. Excess liquid was discarded into sodium hypochlorite. After the inoculum was set, four wells were created in each plate using a sterile cork borer, and the agar plugs were discarded in disinfectant. Each well was sealed with molten agar and filled with different concentrations (500, 250, 125, and 62.5 µg/mL) of Schiff base samples. After allowing 1 hour for pre-diffusion, the plates were incubated at 37 °C for 24 hours against the test organisms. The clinical isolates, as well as the nutrient agar slants, were stored and preserved at 4 °C for short-term preservation and glycerol stocks stored at −20 °C for longer-term maintenance. Zones of inhibition were measured, and the minimum inhibitory concentration (MIC) was noted as the lowest concentration preventing visible growth. The minimum bactericidal concentration (MBC) was determined from MIC tubes showing no growth by transferring their contents to fresh, disinfectant-free media. Lack of regrowth indicated a bactericidal effect, identifying the MBC as the lowest concentration that killed ≥99.9% of the test organisms. A standard ciprofloxacin antibacterial disc was used as a positive control.

LD_{50} Determination

Acute toxicity testing was carried out following Lorke's method [24] using Swiss albino mice. The procedure was conducted in two phases for each compound. In Phase I, nine mice were divided into three groups of three animals each. Each group received intraperitoneal injections of (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol at doses of 10, 100, and 1000 mg/kg, respectively. The mice were monitored for 24 hours for any signs of toxicity or death. In Phase II, three mice were used, with one animal per group. They were given higher doses, 1600, 2900, and 5000 mg/kg, and observed over 24 hours for toxic effects and mortality. The LD_{50} was then calculated using the appropriate formula:

$$LD_{50}=\sqrt{(D_0 \times D_{100})}$$

LD_{50}: median lethal dose; the dose expected to cause death in 50% of the test population.

D_0: highest dose at which no animal died (0% mortality).

D_{100}: lowest dose at which all animals died (100% mortality).

Analgesic Properties Screening

Swiss mice of both sexes, weighing between 16.9 and 25.3 g, were used to evaluate analgesic activity. The test compound, (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol, and the standard drug, pethidine, were administered intraperitoneally to three mice at doses of 25, 50, and 75 mg/kg, respectively. Before administering either the test or the standard drug, each mouse's baseline reaction time was recorded, serving as its control. Following administration, the mice were placed on a hot plate set at 55.0 ± 2 °C, and the time taken to exhibit a pain response, such as paw licking or jumping, was recorded as the reaction time. This measurement was repeated at 30, 60, 90, and 120 minutes post-treatment. Pethidine (12 mg/kg, subcutaneously) served as the reference standard. Reaction times were documented for all animals

following Ghosh. [25] All procedures were reviewed and approved, ensuring compliance with national regulations and promoting responsible scientific practice. Animals were housed under controlled environmental conditions that met requirements for temperature (22–25 °C), humidity (40%–60%), lighting (12-hour light/dark cycle), adequate bedding, and free access to nutrition (pelletized feed and water). Routine health monitoring and husbandry were conducted to maintain consistent welfare throughout the experiment period.

In-Silico Analysis

To validate pharmacological studies, (Z)-4-((4-(dimethylamino) benzylidene)-amino)-phenol and co-crystallized inhibitors of the target enzymes/proteins were subjected to in-silico studies using enzymes and proteins implicated in analgesic and antimicrobial pathophysiology. For the analgesic studies, molecular docking was performed using the COX-1 and COX-2 enzymes, while three enzymes implicated in *E. coli, S. aureus*, and *C. albicans*, respectively, were used for the antimicrobial studies validation. The structure of aspirin acetylated cyclooxygenase-1 (PDB ID: 3N8Y), structure of celecoxib bound to the COX-2 active site (PDB ID: 3LN1), the crystal structure of *E. coli* DNA gyrase B (PDB ID: 7C7N), the crystal structure of penicillin-binding protein (PBP2a) from multi-resistant *S. aureus* (PDB ID: 5M1A), and the crystal structure of sterol 14-alpha demethylase (CYP51) from a pathogenic yeast *Candida albicans* (PDB ID: 5FSA) proteins were utilized for the molecular docking studies. Molecular docking was performed using Glide from the Schrödinger suite, using the parameters of the co-crystallized ligands. [26]

Statistical Data Analysis

All calculations, statistical analysis, and the UV spectrum were constructed using Microsoft Excel 2019. A *P*-value of <0.05 in a given dataset was regarded as significant.

Ethical Approval

The study was approved by the Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria, with approval number EC/FP/025/001 on September 18, 2022.

RESULTS

Synthesis Route

The synthesis of (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol was achieved via the reaction of PAP and cinnamaldehyde, as shown in **Figure 2**.

Physicochemical, NMR, and MS Parameters

The test sample's structural features were characterized with nuclear magnetic resonance (NMR) and Mass spectrometry, respectively. The physicochemical properties, such as actual yield, solubility, melting point, the key ^{1}HNMR, ^{13}CNMR, and MS parameters, are described in **Table 1**, **Figures 3**, and **4**. Paracetamol was used as a comparative reference compound because it shares structural similarity with the synthesized Schiff base precursor (PAP). Its well-characterized UV–visible absorption profile was utilized to ensure calibration accuracy and to provide a benchmark for interpreting the spectral behavior of the synthesized compound.

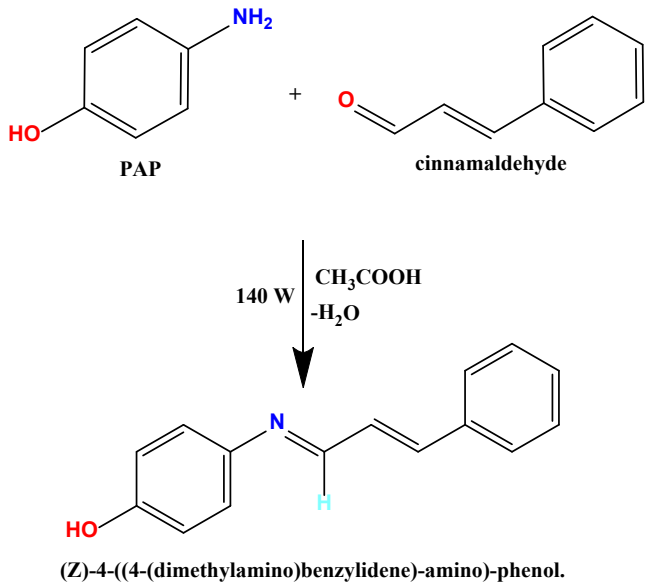

Figure 2: Synthesis of (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol.

UV/Visible Spectrum

The ultraviolet/visible spectroscopic analysis of the test samples is presented in **Figure 4A** and **B**.

Antimicrobial Screening

The MIC of (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol was observed at 250 and 62.5 µg/mL for *E. coli* and *C. albicans,* respectively (**Figure 4C**). 250 µg/mL was observed as the MBC for *E. coli,* respectively (**Figure 4D**). The zone of inhibition is illustrated in **Table 2**.

Acute Toxicity Studies (LD_{50} Determination)

The LD50 was determined to ascertain the acute toxic level of the synthesized compound. The results showed a relative pattern of acute toxicity (**Table 3**).

Analgesic Properties

The analgesic screening was conducted using pethidine as a standard control sample. The results showed promising analgesic activity compared with the standard (**Figure 5A,B**).

Hematological Screening

The hematological parametric analysis was conducted on the test sample. This includes white blood cell (WBC) count, WBC differential analysis, red blood cell (RBC) count, hemoglobin estimation, packed cell volume, and platelet count. Estimation of test sample effect on different hematological parameters, including hemoglobin, packed cell volume, and platelet count, respectively, at various doses (25, 50, and 75 mg/kg) is illustrated in (**Figure 5C–E**). WBC and RBC counts are shown in **Table 3**, while the differential analysis at different doses of WBC is given in **Figure 6**.

Table 1: Solubility profile and TLC analysis of Schiff base derivatives.

S/N	Sample code	Solubility profile						Thin-layer chromatography Rf value (MeOH)		
		MeOH	DMSO	DEE	ACE	RT H_2O	HOT H_2O	1st	2nd	Mean ± SD
	BS8	SS	VS	SS	VS	SS	SS	0.63	0.74	0.69 ± 0.08

NS: not soluble; SS: sparingly soluble; VS: very soluble; H_2O: distilled water; DMSO: dimethyl sulfoxide; DEE: diethyl ether; ACE: acetone; RT: room temperature; MeOH: methanol, solvent system: ethyl acetate: *n*-hexane (1:1); SD, standard deviation; BS8: (Z)-4-((4-(dimethylamino) benzylidene)-amino)-phenol.

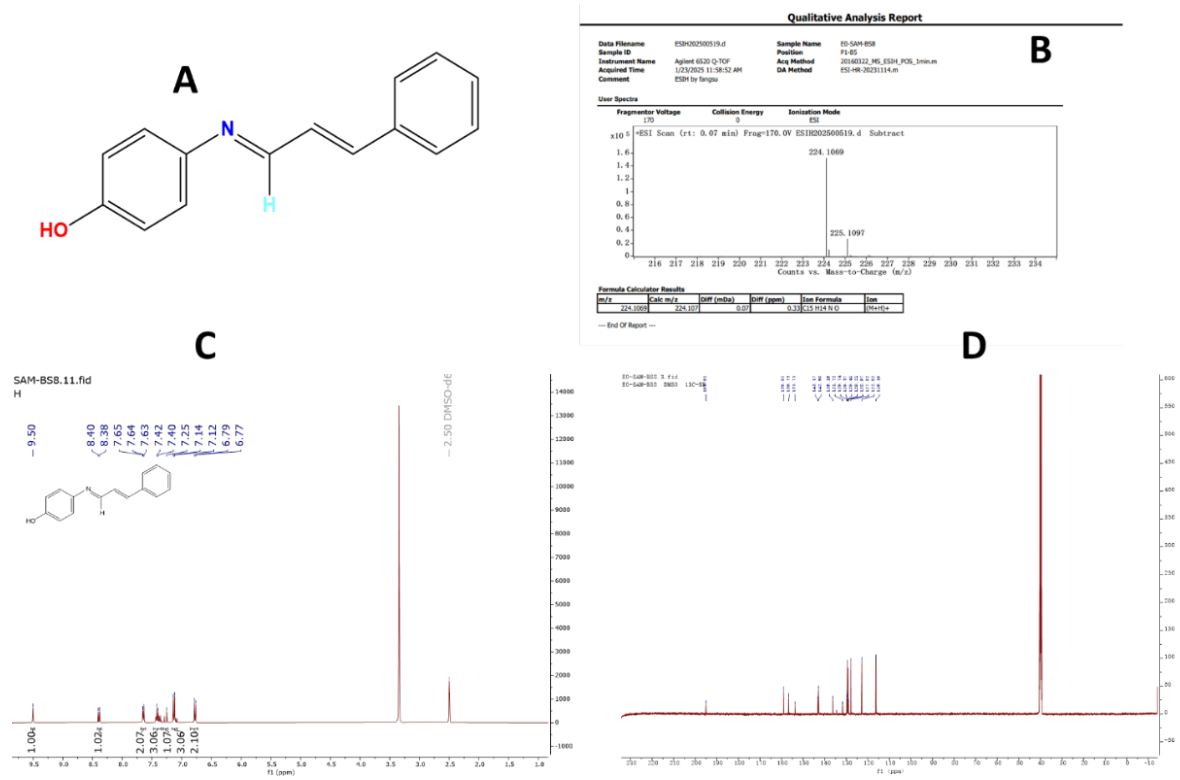

Figure 3: (A) Physicochemical parameters of (Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol: Theoretical yield: 6.3 g, actual yield: 6.012 g, percentage yield: 95.4%, solubility: DMSO, acetone; melting point 192 to 198 °C, LogP: 3.4, LogS: −3.5. (B) 1HNMR spectrum: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.39 (d, *J* = 8.7 Hz, 1H), 7.69 – 7.61 (m, 2H), 7.41 (m, *J* = 7.7 Hz, 3H), 7.25 (s, 1H), 7.13 (d, *J* = 8.7 Hz, 3H), 6.78 (d, *J* = 8.8 Hz, 2H). (C) 13CNMR spectrum: 13C NMR (125 MHz, DMSO-d_6) δ 195.02, 159.02, 156.75, 153.73, 143.17, 142.90, 136.16, 131.72, 129.74, 129.57, 129.41, 129.21, 128.97, 127.82, 122.82, 116.19. (D) HRMS spectrum: HRMS (ESI): C15H14NO [M+H]+ calculated for: 224.1070, found: 224.1069.

Molecular Docking

The molecular docking was performed with a total of five enzymes/proteins associated with analgesic physiology and antimicrobial infection from the organisms used in the study (**Table 4**).

The control compounds: pethidine, aspirin, celecoxib, 6-FMD, ceftazidime, and posaconazole, were used to validate the molecular docking results and test the sample's binding affinity, as well as predict the potential binding target (**Figure 7**).

DISCUSSION

Thesynthesisof(Z)-4-((4-(dimethylamino)benzylidene)-amino)-phenol through microwave-assisted condensation produced a high-yield product with a well-defined physicochemical and spectroscopic profile. The 95.4% yield reflects the efficiency of microwave-assisted synthesis, corroborating previous studies that highlight the benefits of reduced reaction times and higher purity under green chemistry conditions.

The antimicrobial activity observed was moderate, with the most significant inhibition observed against *E. coli* and *C. albicans*. Specifically, *E. coli* showed a 13 mm zone of inhibition at 62.5 µg/mL, while *C. albicans* displayed a 13 mm inhibition zone at 500 µg/mL. The MIC and MBC for *E. coli* were 250 µg/mL, indicating moderate bacteriostatic and bactericidal activity at relatively low concentrations. However, the compound lacked efficacy against *S. aureus* (a gram-positive pathogen), which may suggest specificity toward gram-negative bacteria and fungi. Compared to the

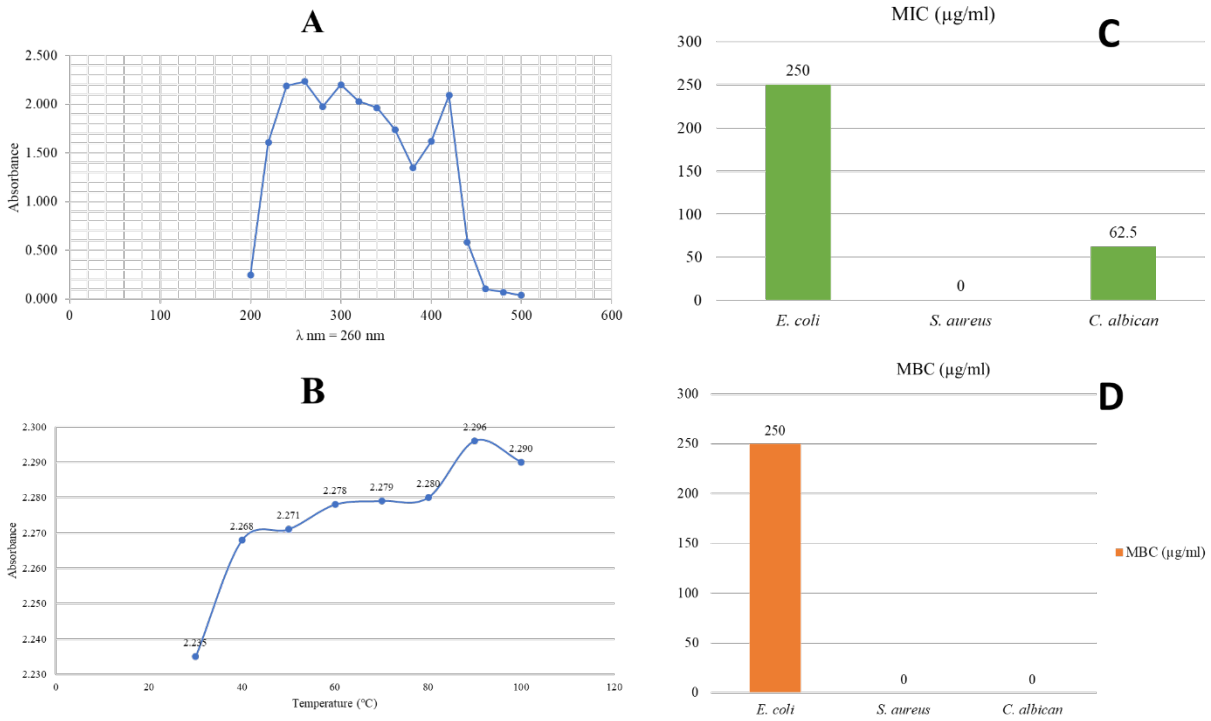

Figure 4: UV spectrum of (A) maximum absorption was observed at 260 nm. Effect of temperature on UV/visible absorbance at 260 nm. (C) Minimum inhibitory concentration. Minimum bacterial concentration (D).

Table 2: Antimicrobial screening results–zones of inhibition.

Sample label	Concentration (µg/mL)	Zone of inhibition (mm)		
		E. coli	*S. aureus*	*C. albicans*
Test sample	500	-	-	13
	250	8.0	-	11
	125	9.0	-	10
	62.5	13.0	-	8
+ve control (CPX)	10 mcg	33.0	29.0	-
+: presence of growth; -: absence of growth; CPX: ciprofloxacin.				

Table 3: Acute toxicity (LD_{50}) determination.

Sample ode	Dose (mg/kg)	Weight of animal (kg)	Dose (mg)	HYP	IMB	SEZ	Death	LD_{50} (mg/kg)
BS8	10	17.9	0.179	0/3	0/3	0/3	0/3	$=\sqrt{(1000 \times 1600)}$ $=1265$
	100	20.2	2.020	0/3	0/3	0/3	0/3	
	1000	21.0	21.00	0/3	0/3	1/3	0/3	
	1600	20.4	32.64	-	-	-	1/1	
	2900	20.1	58.29		-	-	1/1	
	5000	22.0	110.0	-	-	-	1/1	
HYP: hypertension; IMB: immobility; SEZ: seizure. The test compound showed acute toxicity at 1265 mg/kg.								

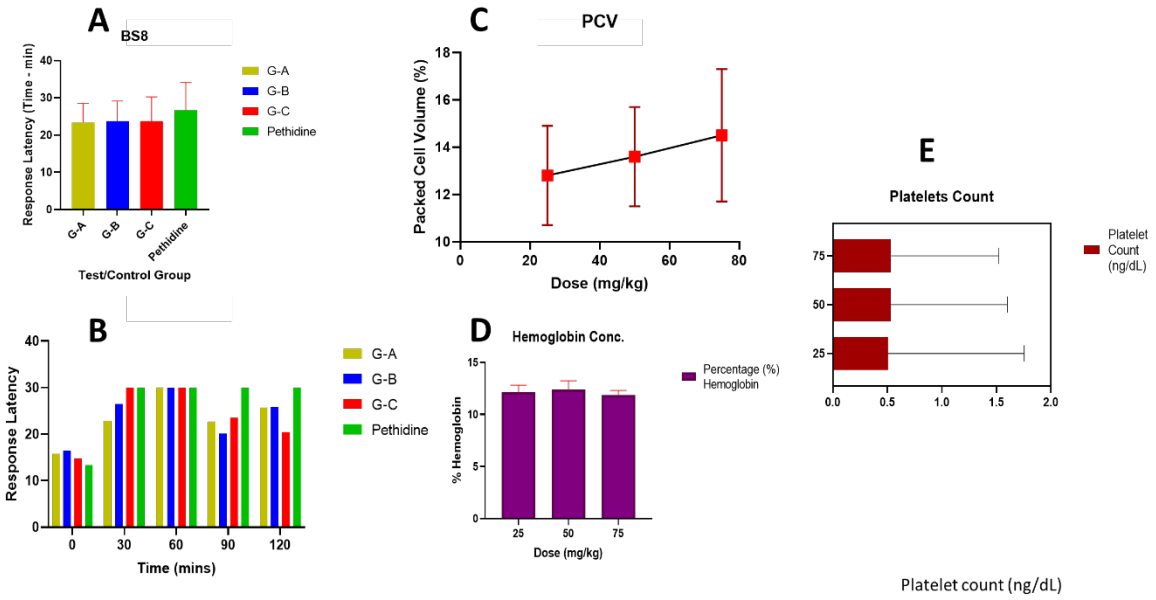

Figure 5: (A) Analgesic activity determination across different time intervals (0–12 minutes), a *P*-value of 0.0002 was obtained as the latency probability from ANOVA, depicting a statistically significant difference. (B) Overall, analgesics were found among the test animal groups compared with the standard (pethidine) group. Similar analgesic activity was observed with a statistically insignificant difference at a *P*-value of 0.80. Effect of test sample on hemoglobin (C), packed cell volume (D), and platelet count (E), respectively, at 25, 50, and 75 mg/kg.

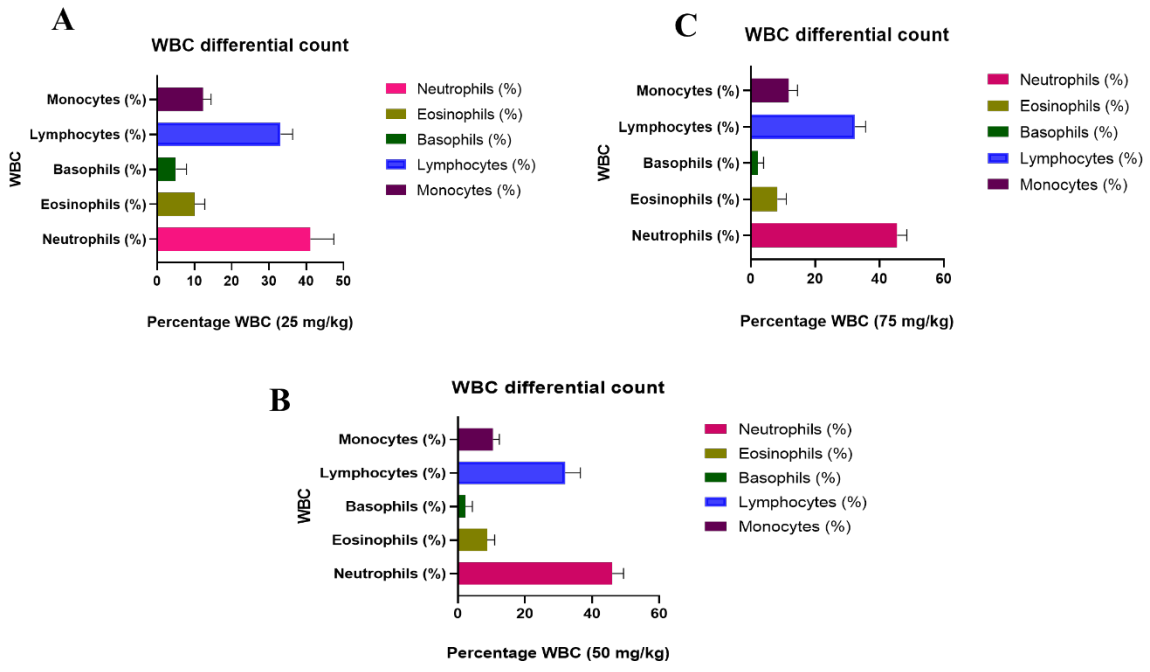

Figure 6: White blood cells (WBC) differential analysis at different doses of test sample: 25 mg/kg (A), 50 mg/kg (B),

ciprofloxacin control (33 mm for *E. coli*, 29 mm for *S. aureus*), the Schiff base is less potent but still demonstrates potential. Compared with previous studies, this compound can be further modified to enhance its bioactivity. [27–32]

Toxicity studies using Lorke's method indicated an LD_{50} of 1265 mg/kg, which places the compound in a moderately toxic range. Observable signs of toxicity included seizures and immobility at higher doses (≥1600 mg/kg), but no fatalities

Table 4: Protein-ligand binding affinity (docking score).

Sample ID	Docking scores (kcal/mol)				
	PDB ID: 3N8Y	PDB ID: 3LN1	PDB ID: 7C7N	PDB ID: 5M1A	PDB ID: 5FSA
Test sample	−6.18	−7.52	−5.41	−6.54	−7.65
Pethidine	-	−6.70	-	-	-
Aspirin	−5.90	−5.88	-	-	-
Celecoxib	-	−11.34	-	-	-
6-FMD	-	-	−7.28	−7.79	−8.72
Ceftazidime	-	-	−6.09	−5.42	−8.21
Posaconazole	-	-	−5.53	−6.61	−11.98

6-FMD: 6-fluoro-8-(methylamino)-2-oxo-1,2-dihydroquinoline.

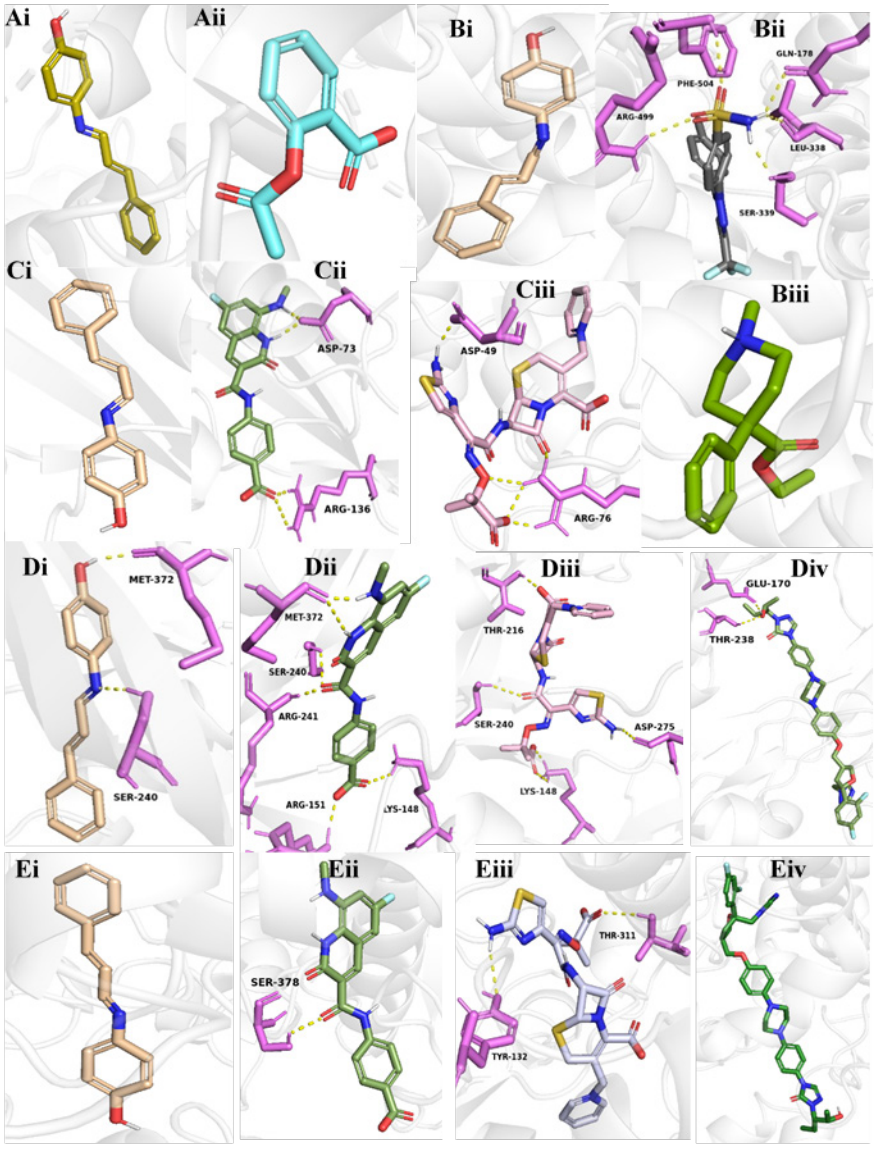

Figure 7: 3D hydrogen-binding interactions of the test sample (Ai) and Aspirin (Aii) with 3N8Y. 3D hydrogen-binding interactions of test sample (Bi), Celecoxib (Bii), Pethidine (Biii) with 3LN1. 3D hydrogen-binding interactions of test sample (Ci), 6FMD (Cii), Ceftazidime (Ciii) with 7C7N. 3D hydrogen-binding interactions of test sample (Di), 6FMD (Dii), Ceftazidime (Diii), Posaconazole (Div) with 5M1A. 3D hydrogen-binding interactions of test sample (Ei) 6FMD (Eii), Ceftazidime (Eiii), Posaconazole (Eiv) with 5FSA. The yellow wedged lines represent H-bonding interactions, while the violet sticks represent the protein amino acid residues.

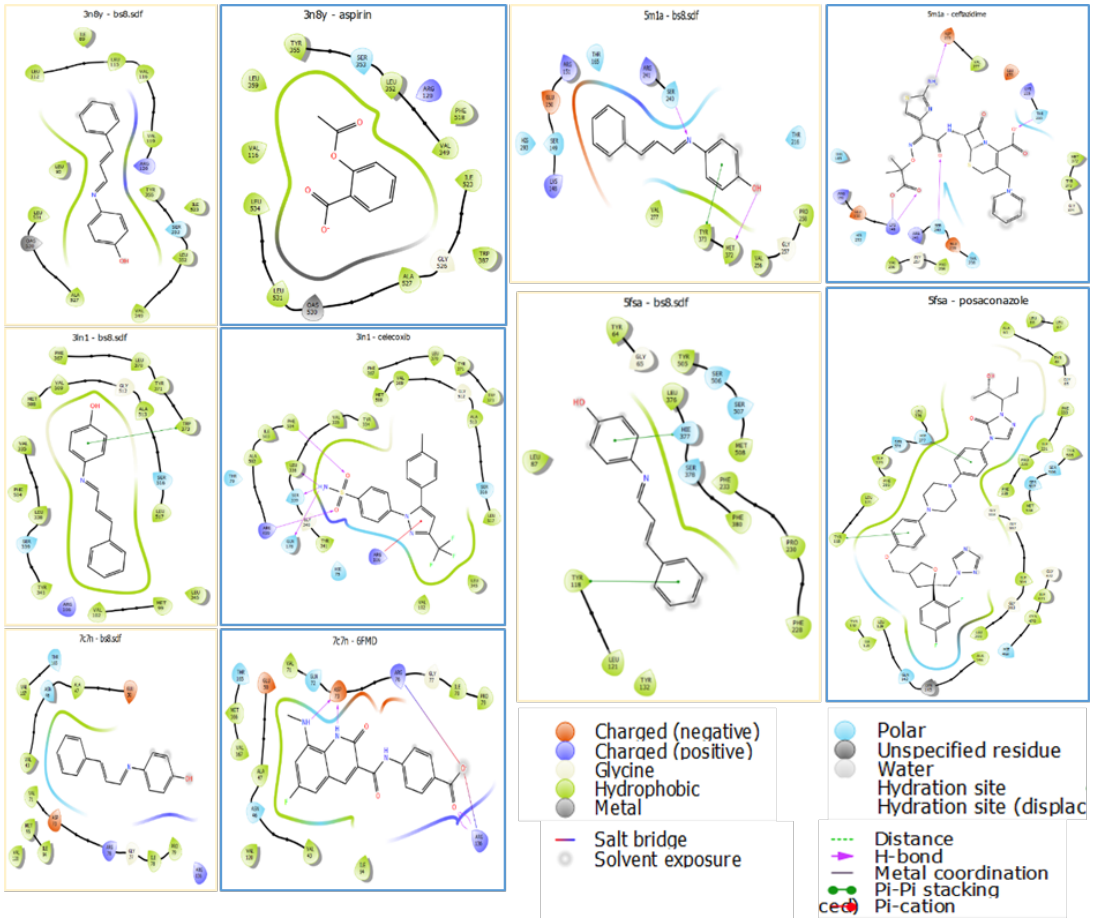

Figure 8: 2D interactions of test sample (BS8) and standards with target proteins.

occurred at 1000 mg/kg, indicating a relatively safe margin for therapeutic application.

The analgesic activity results were particularly compelling. Reaction time significantly increased after administration of the test compound, particularly at 60 and 90 minutes, similar to pethidine, which served as the standard. The statistical analysis (P = 0.0002) confirms the compound's efficacy, and the non-significant difference (P = 0.80) compared to pethidine further validates its analgesic potential. These results align well with the molecular docking data, where strong binding to COX-2 (−7.52 kcal/mol) and COX-1 (−6.18 kcal/mol) was observed, suggesting potential mechanisms for its analgesic effect.

Hematological analysis showed that increasing doses (25, 50, and 75 mg/kg) led to a slight increase in WBC and RBC counts, which may reflect a mild immunostimulatory effect or stress response but remained within physiologically acceptable limits. The WBC and RBC counts suggest no cytotoxic hematological effects at the tested doses. In silico molecular docking showed strong binding to both analgesic and antimicrobial protein targets. Notably, the test compound had a docking score of −7.65 kcal/mol for CYP51 in *C. albicans*, comparable to standard antifungals. Docking to DNA gyrase B in *E. coli* and PBP2a in *S. aureus* also confirmed the potential for antimicrobial activity, though in vitro activity against *S. aureus* was not observed, possibly due to cell wall penetration.

The study demonstrated several strengths. The synthesis of the cinnamaldehyde-derived Schiff produced a high yield of 95.4% through microwave-assisted condensation, which reinforces the advantages of green synthesis. The study also conducted dual pharmacological evaluation, assessing both antimicrobial and analgesic properties. The compound showed measurable antimicrobial activity, particularly against *E. coli* and *C. albicans*, with defined MIC and MBC values, while analgesic testing revealed statistically significant effects comparable to pethidine at peak action. Acute toxicity evaluation provided an LD_{50} of 1265 mg/kg, suggesting moderate toxicity, and hematological assessments indicated no major adverse effects. In silico docking further supported the biological findings, revealing good binding affinities to several analgesic and antimicrobial targets and appropriately comparing the compound's interactions with reference ligands (**Figure 8**).

Despite the promising results obtained, the study had few limitations, including the number of pathogens as only three were tested, chronic toxicity, organ-specific effects, and genotoxicity were not evaluated, analgesic and hematological studies used mice; results may not fully translate to humans, and the lack of experimental enzyme inhibition assays

(COX-2 activity) limits mechanistic validation of molecular docking studies. It is therefore recommended that in future research, antimicrobial screening be expanded to include drug-resistant strains, biofilms, and broader microbial panels, multiple molecules (derivatives) evaluate long-term exposure effects and organ histopathology, conduct absorption, distribution, metabolism, excretion/elimination, toxicity (ADMET) studies and formulation development to improve solubility, perform enzyme inhibition assays (COX-2, 5FSA) to corroborate docking results, modify the Schiff base to enhance *S. aureus* activity and reduce hematological side effects, and investigate the compound's efficacy in infection-induced pain models to validate dual-action potential.

CONCLUSIONS

The Schiff base derivatives, (Z)-4-((4-(dimethylamino) benzylidene)-amino)-phenol, synthesized from cinnamaldehyde and PAP, demonstrate promising antimicrobial and analgesic activities, supported by both experimental and computational data. The compound was effective against *E. coli* and *C. albicans*, with MIC and MBC values indicating bacteriostatic and bactericidal properties, respectively. Analgesic testing using the hot-plate method and the reference standard (pethidine at 12 mg/kg) confirmed statistically significant pain relief, similar to pethidine, and molecular docking supported these findings with high affinity for COX-2 and 5FSA, among other targets. With an LD_{50} of 1265 mg/kg, the compound can be classified as moderately toxic, but no severe hematological toxicity was observed at therapeutic doses. The hematological profile remained within normal physiological ranges, supporting the compound's in vivo safety at lower doses. Overall, the compound shows dual pharmacological potential, and with further optimization and derivatization, it may serve as a lead compound for the development of new antimicrobial and analgesic drugs.

AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

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LIST OF ABBREVIATIONS

4CL	4-coumarate-CoA ligase
ADMET	absorption, distribution, metabolism, excretion/elimination, toxicity
COX	cyclooxygenase
GSH	glutathione (reduced)
LD_{50}	lethal dose (acute toxicity)
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
PAL	phenylalanine-ammonia-lyase
PAP	*para*-aminophenol
PDB	Protein Data Bank
RBC	red blood cell
TLC	thin-layer chromatography
WBC	white blood cell

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