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Synthesis, Characterisation and Antimicrobial Evaluation of 4-Bromo-2-[(Dodecylimino) Methyl] Phenol and 4-Bromo-2-[(Hexadecylimino) Methyl] Phenol

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ABSTRACT

Schiff bases were synthesized by the condensation of 5-bromosalicylaldehyde with hexadecylamine (5-Br SA-HA) and dodecylamine (5-Br SA-DA). The resulting compounds were characterized using elemental analysis, mass spectrometry, ultraviolet-visible (UV-Vis) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopic techniques. The antimicrobial activities of the synthesized Schiff bases were evaluated against four bacterial strains, Gram-positive *(Bacillus subtilis* (*B. subtilis*)) and Gram-negative (*Pseudomonas aeruginosa* (*P. aeruginosa*)), as well as two fungal strains, *Aspergillus niger* (*A. niger*) and *Penicillium chrysogenum* (*P. chrysogenum*).

Keywords: Schiff bases, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Penicillium chrysogenum*.

INTRODUCTION

The increasing prevalence of drug-resistant bacteria and fungi poses a significant risk to patients, healthcare systems, and the global economy [1-2]. Therefore, there is an immediate necessity to create innovative drugs that operate through novel mechanisms to effectively counteract these pathogens.

Schiff base ligands have been widely investigated for their antimicrobial activity over the past several decades.[3-7]. An important factor underlying this biological activity is the presence of the azomethine group (–C=N–), which is thought to play a crucial role in interacting with microbial cell components [8-9]. Salicylaldehyde and its substituted derivatives have been reported to possess antimicrobial properties [10-11]. Likewise, long-chain alkylamines like dodecylamine and hexadecylamine have demonstrated notable antimicrobial activity [12-14]. Therefore, Schiff bases resulting from the condensation of salicylaldehyde derivatives with long-chain alkylamines emerge as promising candidates for antimicrobial applications.

Herein, we report the synthesis of 5-Br SA-DA and 5-Br SA-HA from 5-bromosalicylaldehyde with dodecylamine and hexadecylamine, respectively, and the evaluation of the compounds against two bacterial strains - *B. subtilis* and *P. aeruginosa*, along with two fungal strains, *A. niger* and *P. chrysogenum*.

MATERIALS AND METHODS

Chemicals

Methanol, hexadecylamine, dodecylamine and salicylaldehyde were of analytical grade and used without further purification.

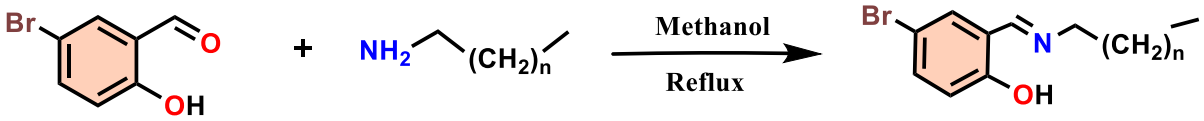
Characterization Method

Elemental analysis was carried out using an Elementar Vario EL III CHN analyser. Absorption spectra were recorded on Shimadzu UV-3600 UV-VIS-NIR. ^{1}H and ^{13}C NMR spectrum was obtained on a Brucker Avance III HD 500 spectrometer. Mass spectra were obtained using a Bruker UltrafleXtreme MALDI-TOF/TOF mass spectrometer with Matrix-Assisted Laser Desorption/Ionisation (MALDI).

Synthesis of Schiff base ligands (5-Br SA-DA and 5-Br SA-HA)

The Schiff base ligands were prepared by the condensation reaction of 5-bromosalicylaldehyde with amines.[15]. A solution of the corresponding amine (5 mmol) in 5 mL of methanol was added dropwise to a 5 mL methanolic solution containing 5 mmol of 5-bromosalicylaldehyde. The reaction mixture

was then refluxed at 100 °C under constant stirring for 10 hours (Scheme 1). After cooling to room temperature, the product was either poured onto ice and recrystallised from alcohol (for 5-Br SA-HA) or extracted with hexane (for 5-Br SA-DA).



n=14, 5-Br SA-HA
n=10, 5-Br-SA-DA

Scheme1. Synthesis of 5-Br SA-DA and 5-Br SA-HA

Antimicrobial Activity by Agar Disc Diffusion Method

The antimicrobial effects of 5-Br SA-HA and 5-Br SA-DA were investigated in vitro using the agar disc diffusion method against four bacterial (*S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*) and two fungal strains (*A. niger* and *P. chrysogenum*).

Antimicrobial activity was evaluated using the disc diffusion method [16] with slight modifications. To prepare lawn cultures, 0.1 mL of each bacterial suspension or fungal spore suspension was evenly spread onto nutrient agar plates. Sterile filter paper discs (4 mm in diameter) were loaded with different concentrations of the test ligands (5, 10, or 20 µL of a 10 mg/mL chloroform solution) and placed on the agar surface at 2 cm intervals using sterile forceps. A chloroform-treated sterile disc, dried prior to use, served as the negative control. The inoculated plates were incubated at 37 °C for 24 hours for antibacterial assessment and at room temperature for four days for antifungal activity. After incubation, the diameters of the zones of inhibition were measured in millimetres.

RESULTS AND DISCUSSION

Characterization of 5-Br SA-DA and 5-Br SA-HA

The synthesised ligands were characterised using elemental analysis, mass spectrometry, UV-Vis and ^{1}H NMR spectroscopic techniques.

Elemental Analysis

The analysis results of 5-Br SA-DA and 5-Br SA-HA were in close agreement with the empirical formula as shown in Table 1.

Table 1: Physical and analytical data of the synthesised ligands

Ligand	**Empirical Formula**	**Formula Weight**	**Colour**	**(Found) Calculated %**		
			(Yield %)	**C**	**H**	**N**
5-Br SA-DA	$C_{19}H_{30}BrNO$	368.35	Brown (68)	(62.23) 61.90	(9.97) 9.71	(4.49) 4.78
5-Br SA-HA	$C_{23}H_{38}BrNO$	424.46	Yellow (84)	(64.93) 65.08	(9.28) 9.02	(3.61) 3.30

MALDI-TOF

The MALDI-TOF mass spectra of the ligands were recorded and are presented in Figure 1. The calculated and observed m/z ratios of the $[M + H]^+$ ions for the ligands were compared and are summarized in Table 2. These findings validate the effective formation of the corresponding ligands.

Table 2: Calculated and found m/z ratio for $[M +H]^+$ ions of ligands

Ligand	**Molecular Formula**	**m/z ratio of $[M +H]^+$ ions**	
		Calculated	**Observed**
5-Br SA-DA	$C_{19}H_{30}BrNO$	367.151	367.713
5-Br SA-HA	$C_{23}H_{38}BrNO$	423.213	423.550

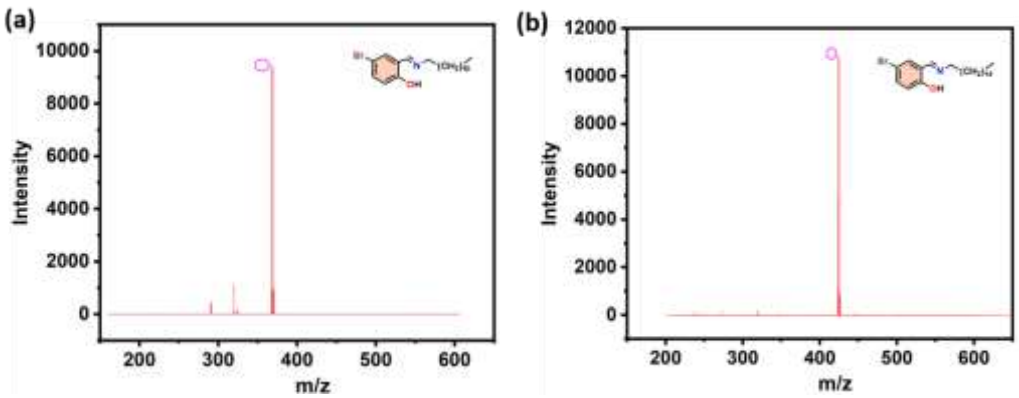


Figure 1: The mass spectra of (a) 5-Br SA-DA (b) 5-Br SA-HA.

UV-Vis Spectroscopy

The UV-Vis spectra of the compounds were measured in chloroform and are shown in Figure 2. The peaks at approximately 327 nm and 328 nm correspond to the n→π* transition associated with the C=N group for 5-Br SA-DA and 5-Br SA-HA, respectively. Another peak at around 253 nm is attributed to the π→π* transition of the aromatic ring.

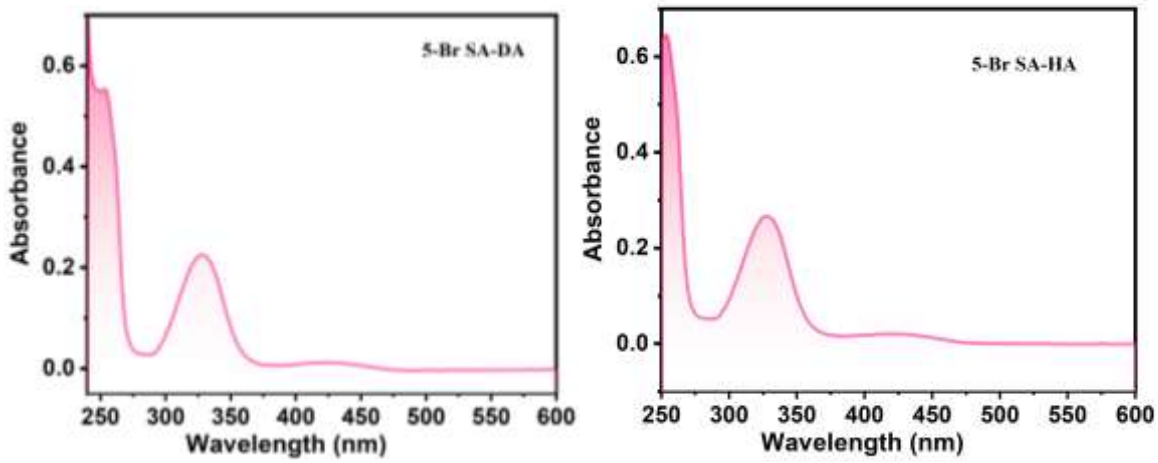


Figure 2: UV-Vis spectra of (a) 5-Br SA-DA (b) 5-Br SA-HA

^{1}H NMR

^{1}H NMR spectrum of 5-Br SA-DA and 5-Br SA-HA were recorded with $CDCl_3$ as solvent and is depicted in Figures 3 and 4. The singlet peak at δ 8.28 ppm and 8.25ppm for 5-Br SA-DA AND 5-Br SA-HA, respectively, corresponds to the azomethine (–CH=N–) proton. The aromatic protons resonate in the region of 7.29-6.75 ppm and appear as a multiplet. A triplet in the upfield region 0.81-0.78 ppm indicates the terminal methyl group. The methylene protons adjacent to azomethine nitrogen ($–CH_2–N=C–$) give rise to a triplet in the region 3.64-3.57 ppm. The remaining methylene protons appear as mutiplet in the region of 1.63-1.17 ppm. The spectral analysis verifies the successful formation of the imine compounds.

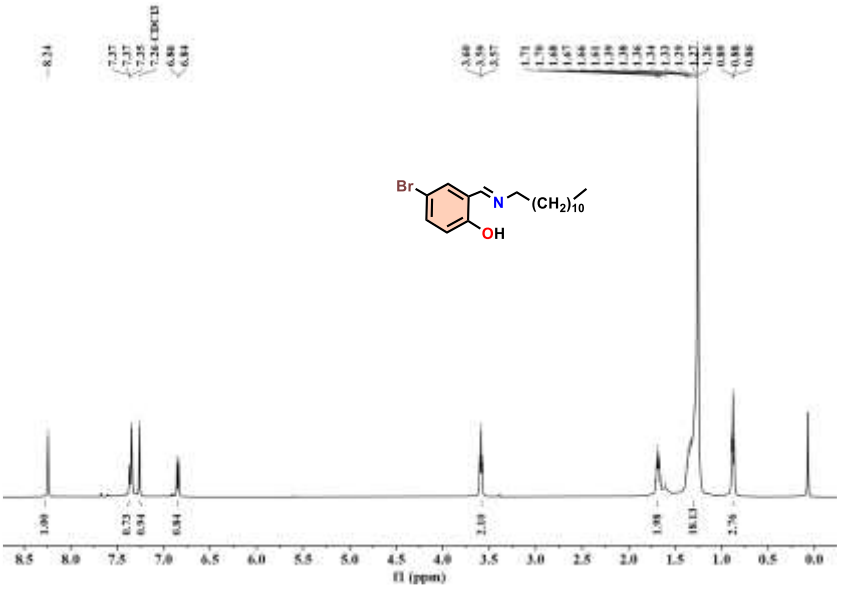


Figure 3: ^{1}H NMR spectra of 5-Br SA-DA.

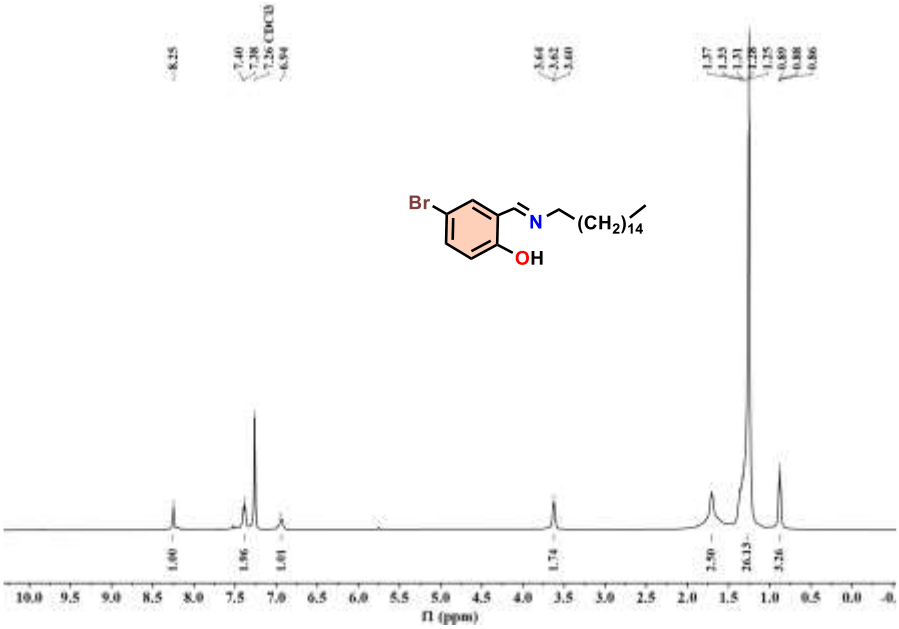


Figure 4: ^{1}H NMR spectra of 5-Br SA-HA.

ANTIBACTERIAL STUDY

The diameters of the inhibition zones of 5-Br SA-DA and 5-Br SA-HA against *P. aeruginosa* and *B. subtilis* were measured and are presented in Table 3. Photographic images depicting the zones of inhibition are provided in Figure 5.

Table 3: Inhibition zone diameters of 5-Br SA-DA and 5-Br SA-HA against *P. aeruginosa* and *B. subtilis*

Compound	***Zone Diameter (mm)***					
	P. aeruginosa			***B. subtilis***		
	5 µL	10 µL	20 µL	5 µL	10 µL	20 µL
5-Br SA-DA	0	8	18	8	10	10
5-Br SA-HA	10	14	20	0	6	12

*The measured zone of inhibition values include the diameter of the disc (4 mm).

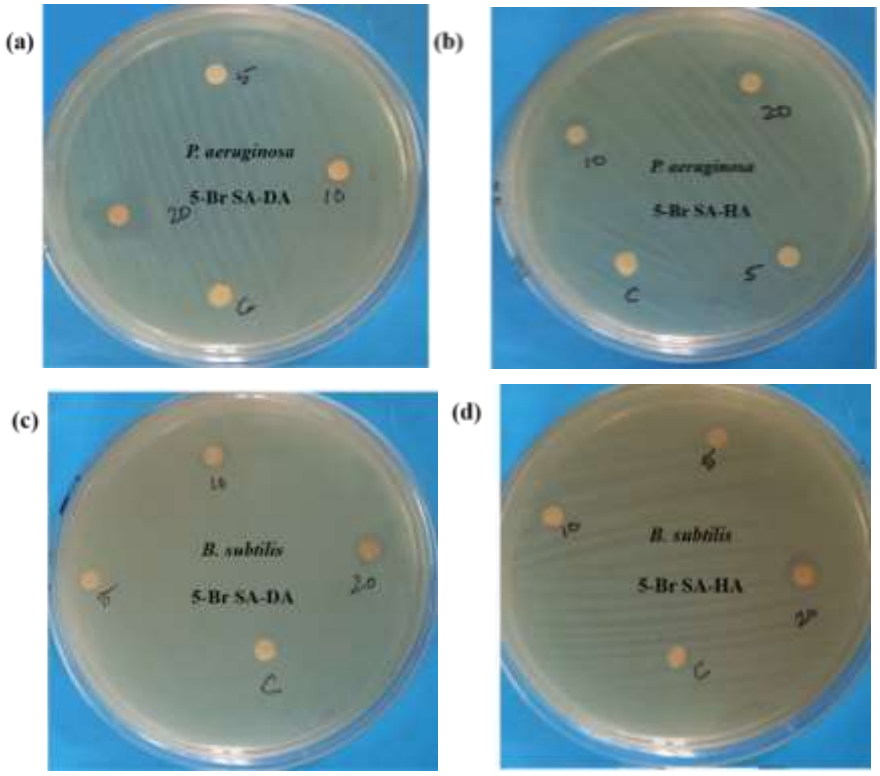


Figure 5: Inhibition zones produced by 5-Br SA-DA and 5-Br SA-HA against *P. aeruginosa* (a, b) and *B. subtilis* (c, d). Sample 'c' represents the control.

A dose-dependent enhancement in activity is observed for both compounds against the bacterial strain *P. aeruginosa*. In contrast, against *B. subtilis*, 5-Br SA-HA exhibits a similar dose-response trend, whereas 5-Br SA-DA displays comparable activity at both 10 µL and 20 µL doses. 5-Br SA-HA is found to be more potent than 5-Br SA-DA against both bacterial strains. An increase in activity is observed with an increase in chain length of the alkyl amine moiety of the Schiff base [17].

ANTIFUNGAL STUDY

The diameters of the inhibition zones of 5-Br SA-DA and 5-Br SA-HA against *A. niger* and *P. chrysogenum* were measured and are presented in Table 4. Photographic images depicting the zones of inhibition are provided in Figure 6.

Table 4: Inhibition zone diameters of 5-Br SA-DA and 5-Br SA-HA against A. *niger* and *P. chrysogenum*

Compound	***Zone Diameter (mm)***					
	A. niger			***P. chrysogenum***		
	5 µL	10 µL	20 µL	5 µL	10 µL	20 µL
5-Br SA-DA	0	8	12.4	6	10	12
5-Br SA-HA	0	6	14	0	6	12.2

*The measured zone of inhibition values include the diameter of the disc (4 mm).

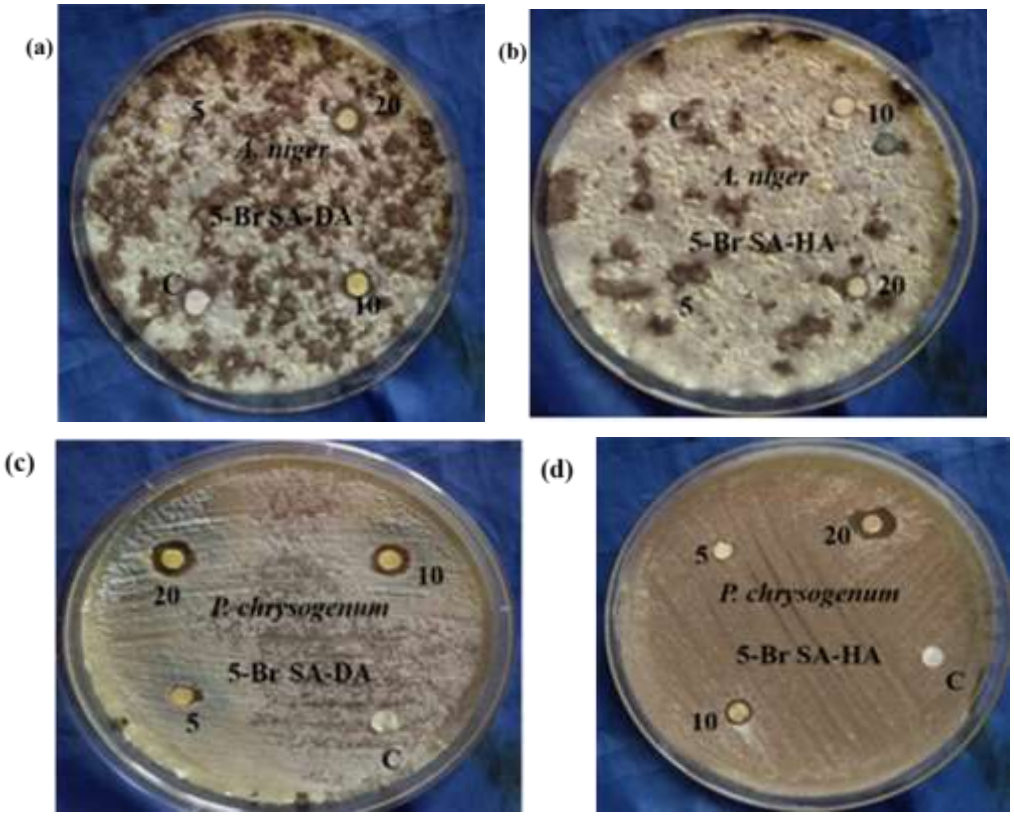


Figure 6: Inhibition zones produced by 5-Br SA-DA and 5-Br SA-HA against *A. niger* (a, b) and *P. chrysogenum* (c, d). Sample 'c' represents the control.

A similar trend is observed in the antifungal property of these compounds; a dose-dependent increase is seen in both compounds. Greater antifungal potency is observed for 5-Br SA-HA than 5-Br SA-DA. As chain length increases, an increase in antifungal property is observed. A similar trend is noted in the antifungal activity of these compounds, with both showing a dose-dependent enhancement. Among them, 5-Br SA-HA exhibits stronger antifungal efficacy compared to 5-Br SA-DA. The antifungal activity improves with the elongation of the alkyl chain [18].

CONCLUSION

Two Schiff bases, 5-Br SA-DA and 5-Br SA-HA, were synthesised via the condensation reaction of 5-bromosalicylaldehyde with dodecylamine and hexadecylamine, respectively. The formation of the imine (C=N) functional group was confirmed by elemental analysis, mass spectrometry, UV-Vis, and ^{1}H NMR spectroscopy, all of which supported the formation of the characteristic C=N bond. The antimicrobial activities of the synthesised Schiff bases were evaluated against two bacterial strains (P. *aeruginosa* and *B. subtilis*) and two fungal strains (*A. niger* and *P. chrysogenum*). Both Schiff bases demonstrated significant antimicrobial effects, which increased in a dose-dependent manner. Furthermore, the antimicrobial potency improved with the elongation of the aliphatic chain.

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