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ANTIMICROBIAL ACTIVITY OF AZO-SCHIFF BASES DERIVED FROM SALICYLALDEHYDE AND *PARA*-SUBSTITUTED ANILINE

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ABSTRACT

This study was designed to evaluate the antimicrobial activity of azo-Schiff bases (4-[(*E*)-(4-methylphenyl)diazenyl]-2-{[(4-nitrophenyl)imino]methyl}phenol (ASBn) and 4-[(*E*)-(4-methylphenyl)diazenyl]-2-{[(4-methylphenyl)imino]methyl}phenol (ASBm), prepared by the condensation of azo salicylaldehyde and para-substituted aniline. The azo-Schiff bases were characterized by IR spectroscopy and C, H, N analytical data. The antimicrobial activities of synthesized Schiff bases were determined in terms of zones of inhibition and minimum inhibitory concentrations (MICs). All the compounds showed moderate to good activities against all the tested microorganisms with MICs 50-500 mg/ml against different microorganisms. The electron withdrawing substituent on the azo Schiff bases showed enhanced antimicrobial activity than electron donating substituent. These Schiff bases may prove to be potential candidates for future antibiotic drugs.

KEYWORDS: Salicylaldehyde, Schiff bases, Azo, antifungal, antibacterial, aniline.

INTRODUCTION

Schiff bases are a product of condensation between aldehydes and primary amines. They possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and synthetic flexibility that enables design of suitable structural properties. Schiff bases have been shown to be promising leads for the design of efficient antimicrobial agents as a result of their broad range of biological activities. These biological activities include: antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic, anti-hypertensive, herbicidal, anti-convulsant,

anti-oxidant, anti-tumor, anti-depressant and cytotoxic activities.[1-14] Schiff bases are also used as pigments and dyes, catalysts and intermediates in organic synthesis and as polymer stabilizers.[12]

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistances to antibiotics. The lack of effective treatments has been noted as the main cause of this problem.[15-16] The development of new antibacterial and antifungal agents with novel and more efficient mechanisms of action is definitely an urgent medical need.[17-18] In view of these facts, this study is aimed at evaluating the antibacterial and antifungal activities of two azo Schiff bases derived from salicylaldehyde.

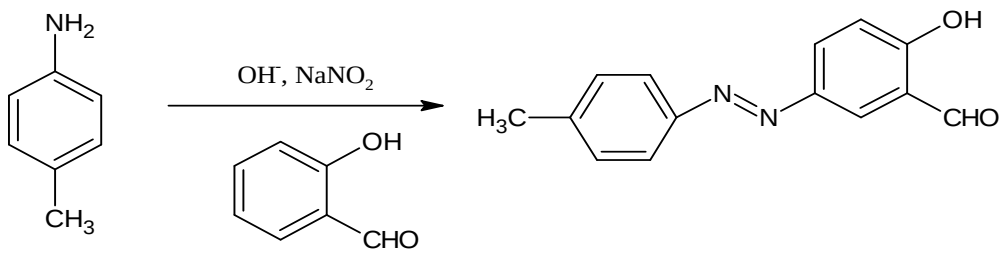
MATERIALS AND METHODS

General

All chemicals used were of analytical grade (Sigma-Aldrich) and were used without further purification. The IR spectra were recorded on a Shimadzu FTIR-IR Prestige-21 (200VCE) spectrophotometer as KBr pellets between 4000-400 cm^{-1}. The C, H, N data were determined using a Perkin-Elmer instrument model 240B.

Synthesis of azo salicylaldehyde

Azo compound was prepared by a method reported earlier.[19] A solution of HCl was prepared by diluting 5 ml of concentrated HCl acid in 20 ml of distilled water. 10 mmole of p-toluidine was added, stirred to dissolved and cooled to 0^oC. 8 ml of aqueous $NaNO_2$ (10 mmole) was added in drop while maintaining the temperature at $0\text{-}5^oC$. The diazonium chloride formed was consecutively coupled with 10 mmole of salicylaldehyde (1.0656 ml) that was dissolved in an aqueous solution containing 50 ml of 0.4 g NaOH and 7.39 g of Na_2CO_3. The reaction mixture was stirred at 0^oC for one hour. The precipitated product was filtered, recrystallized from ethanol and weighed. Colour: brown; Yield: (1.74 g) 72 %; IR (KBr cm-1): 3612-3381 (Ar-OH); 1453 (N=N, azo), 1493 (C-C, benzene), 3053 (C-H, benzene); Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C 66.99, H 5.03, N 11.66; found C 69.61, H 4.64, N 11.27.



Scheme 1. Preparation of azo salicylaldehyde.

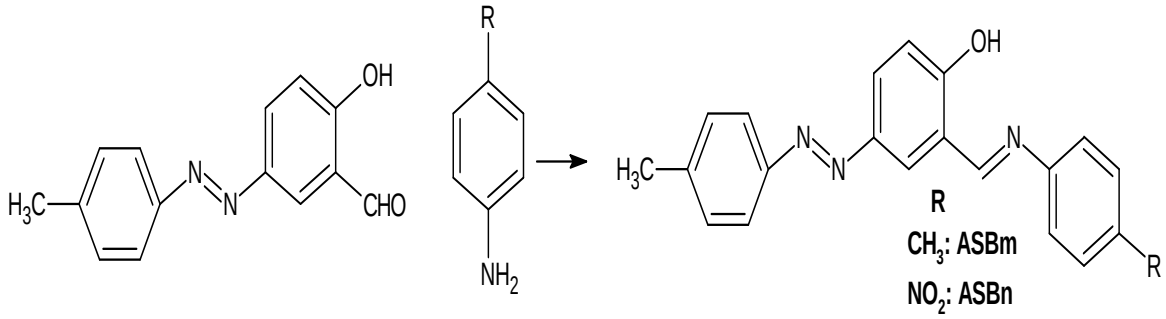
Synthesis of azo Schiff bases

4-[(*E*)-(4-methylphenyl)diazenyl]-2-{[(4-nitrophenyl)imino]methyl}phenol (ASBn)

The of azo salicylaldehyde (4 mmole) was dissolved in 30 ml of ethanol and p-nitroaniline (4 mmole) was also dissolved in 30 ml of ethanol separately. The two ethanolic solutions were mixed together. The mixture was heated at reflux with stirring for four hours. The precipitated product was filtered, recrystallized from ethanol and suction dried. Colour: dark brown; Yield: (0.98 g) 68 %; IR (KBr cm^{-1}): 3613-3381 (Ar-OH); 1451 (N=N, azo), 1495 (C-C, benzene), 3051 (C-H, benzene); Anal. Calcd. for $C_{20}H_{16}N_4O_3$: C 66.66, H 4.48, N 15.55; found C 66.28, H 4.09, N 15.17.

4-[(E)-(4-methylphenyl)diazenyl]-2-{[(4-methylphenyl)imino]methyl}phenol (ASBm)

The azo salicylaldehyde (4 mmole) was dissolved in 30 ml of ethanol, and p-toluidine (4 mmole) was also dissolved in 30 ml of ethanol separately. The two ethanolic solutions were mixed together. The mixture was heated at reflux with stirring for four hours. The precipitated product was filtered, recrystallized from ethanol and suction dried. Colour: light brown; Yield: (0.94 g) 71 %; IR (KBr cm^{-1}): 3612-3382 (Ar-OH); 1452 (N=N, azo), 1491 (C-C, benzene), 3052 (C-H, benzene); Anal. Calcd. for $C_{21}H_{19}N_3O$: C 76.57, H 5.81, N 12.76; found C 76.18, H 5.42, N 12.38.



Scheme 2. Preparation of azo Schiff bases.

Collection and preparation of bacterial and fungus Isolates

Clinical bacterial and fungus isolates were collected from infected wounds using sterile swab sticks at St. Lukes Hospital, Anua, Uyo and Macson's clinic, Ukanafun Local Government Area, Akwa Ibom State. These isolates were transported on slants to Microbiology Laboratory, University of Uyo. The test organisms were sub-cultured into nutrient broth and incubated for 48 hrs at 37ºC. The microbes were sub cultured on nutrient agar slant for the isolation of pure culture. Isolates were identified using standard cultural, microscopic and

standard biochemical methods such as motility test, gram staining, oxidase test, oxidation fermentation test, indole test, catalase test, gelatin liquefaction test, citrate utilization, esculin hydrolysis, urease activity, decarboxylase reactions and hydrogen sulphide production tests. The Gram positive bacteria, *Staphylococcus aureus* and *Streptococcus agalactiae*; fungus, *Candida vulgaris* were serially diluted to factor three using 10 fold dilution. Gram negative isolates (*Pseudomonas aeruginosa, Kiebsiella pneumonia* and *Proteus mirabilis*) were serially diluted to factor five using 10 fold dilution. The isolates were sub-cultured into their selective medium based on their exhibited morphological characteristics. They were preserved at 4°C and later used for this work.

Preparation of antimicrobial discs

A 5 mm diameter plunger was used to punch a Whatman no.1 absorbent filter paper to obtain 5 mm diameter paper discs. The discs were properly labeled for identification purposes and then sterilized by autoclaving for 15 min at 121°C. The disc were impregnated with different concentrations (0.1-0.4g/ml) of the azo Schiff bases, dried and stored off in sterile bottles.

Evaluation of antimicrobial activity

Antimicrobial activity was tested using a modified discs diffusion assay (DDA) method.[20] The inoculums for each microorganism were prepared from broth cultures. A loop of culture from the nutrient agar (NA) slant stock was cultured in Mueller Hinton medium overnight and spread with a sterile swab into Petri-plates. Each microbial swab was spread on separate plates. Sterile disc (5 mm in diameter) impregnated with different concentrations of the azo Schiff bases were placed on the cultured plates. Control experiment was carried out using commercial antibiotics, antifungal and solvent (stock). The solvent loaded disc without compound served as negative control in the study. Streptomycin (80mg/ml) was used for bacterial isolates and Nystatin (150mg/ml) for fungus isolates; plates were incubated for 24 hours and 48 hours respectively. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate readings.

Determination of minimum inhibitory concentrations (MIC)

The MIC of each azo Schiff base was determined using tube dilution method.[21-22] Different concentrations of each compound were prepared and standard volume (0.1 ml) of each isolate was aseptically inoculated into different concentrations of the azo Schiff base. Control experiment was carried out without the compounds. All tubes were incubated at 37°C for 24

hours. Minimum inhibitory concentrations were determined as the lowest concentration of the compounds that showed no turbidity.

RESULTS AND DISCUSSION

The Schiff bases ASBn and ASBm were prepared as shown in Scheme 2. The *in vitro* antimicrobial activities of the Schiff bases against wound pathogens were evaluated in terms of zones of inhibition and MIC values and the results are presented in Table 1 and Table 2, respectively. The different pathogens showed varied sensitivities in the assay. The zone of inhibitions for the Gram-positive bacteria, Gram-negative bacteria and fungus were 8.0-14.0 mm; standard drug, streptomycin (12-26mm) and nystatin (15mm). These drugs were used as standards to have a comparison of the inhibitory effects of the synthesized Schiff bases.

Table 1. Antimicrobial activity (zones of inhibition in mm) of azo-Schiff bases derived from salicylaldehyde.

Microbial strains	ASBn (mg/ml)				ASBm (mg/ml)				Standards (mg/ml)	
	100	200	300	400	100	200	300	400	Streptomycin	Nystatin
S. aureus	9	10	12	13	--	--	8	9	26	NT
S. agalactiae	8	10	12	12	--	--	9	10	12	NT
P. aeruginosa	8	9	12	13	--	--	--	--	17	NT
K. pneumonia	--	--	12	13	8	10	12	12	16	NT
P. vulgaris	--	9	11	12	--	--	--	10	14	NT
C. vulgaris	8	12	13	14	--	--	12	13	NT	15

NT: Not tested; --: No observable inhibition.

ASBn demonstrated better antimicrobial potency compared to ASBm, except for *K. pneumonia* at lower concentrations in the study. At these relative low concentrations of 100-200mg/ml, the Gram-positive bacteria were more susceptible to the Schiff bases than the Gram negative bacteria. The morphology of the cell wall is a key factor that influences the activity of antibacterial agents. It has been suggested, that Gram-positive bacteria are more sensitive to chemical compounds than Gram negative bacteria due to the relative thickness of their cell walls.[23] Generally, *C. vulgaris* was observed to show the most sensitivity in the assay (14mm, 400mg/ml). However, a better understanding of antimicrobial inhibitions is reflected in MICs. The minimum inhibitory concentration gives approximation to the least concentration of an antimicrobial needed to prevent microbial growth.

Table 2: Minimum inhibitory concentration of azo-Schiff bases derived from salicylaldehyde.

Microbial strains	ASBN (mg/ml)	ASBM (mg/ml)
S. aureus	50	250
S. agalactiae	50	250
P. aeruginosa	50	500
K. pneumonia	250	50
P. vulgaris	150	350
C. vulgaris	50	250

The MIC result in Table 2 is a reflection of the substitution effect (-NO_2 and -CH_3) on the antimicrobial activity of the studied Schiff bases (MIC for ASBn, 50.0-250 mg/ml and ASBm, 50.0-500mg/ml). The highest MIC in the assay was observed for *P. aeruginosa*. Antimicrobial activity depends on the nature of bacterial strain, the solvent and chelating ability of the Schiff base. It is believed that Schiff bases act by forming a chelate with the bacterial strain. This may involve hydrogen bonding through the azomethine group with the active centres of cell constituents thus resulting in an interference with normal cell process.[24] Hence, the better the hydrogen bonding ability, the more active the compound. The antimicrobial activity of Schiff bases derived from aminobenzoic acids in different solvents (MIC, 3.125-25mg/ml) and salicylaldehyde with different aliphatic amines (MIC, 50-250 µg/ml) have been reported to show varying degree of microbial susceptibility, attributed to structural differences and solvent effect.[25-26] Schiff bases with a 2,4-dichloro-5-fluorophenyl moiety have also been shown to be effective in the inhibition of bacterial growth. Schiff bases from this class of compound inhibited the growth of *S. aureus*, *Escherichia coli*, *P. aeruginosa*, and *K. pneumonia* with MIC values ranging from 6.3 to 12.5µg/ml, which are comparable to those obtained for the reference drug, ciprofloxacin.[27] The 5-chloro-salicylaldehyde-Schiff base derivatives have also been shown to be most active against bacterial species, *P. fluorescence*, *E. coli*, *Bacillus subtilis* and *S. aureus* (MIC, 1.6-5.7 µg/ml).[28] The results obtained from the present work shows that the antimicrobial activities of the studied Schiff bases depend on the molecular structure. The ASBn possesses –NO_2 which is electron withdrawing whereas ASBm possesses –CH_3 which is electron donating substituent. The electron withdrawing substituent shows higher antimicrobial activities than the electron donating substituent.

CONCLUSIONS

The Schiff bases, ASBn and ASBm exhibited broad spectrum *in vitro* antimicrobial activity against the pathogens isolated from infected wounds. ASBn showed better antibacterial and antifungal activity than ASBm. The activity data suggest that the antimicrobial activity is dependent on the molecular structure of the compound. The electron withdrawing substituent exhibits better antimicrobial activity compared to electron donating substituent. These Schiff base compounds may prove to be promising leads for the design of more efficient antimicrobial agents.

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