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Anti-Salmonellal Schiff Bases from Vanillin: Synthesis and Structure Elucidation

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Abstract

A series of vanillin substituted Schiff bases namely; 2-methoxy-4-(pyridine-3-yl-hydrazonomethyl)-phenol, NT_1 (1), (4-hydroxy-3-methoxybenzylidene) isonicotinohydrazide, NT_2 (2), 4-[(2,4-dinitrophenyl) hydrazonomethyl]-2-methoxyphenol, NT_3 (3), 2-methoxy-4-phenyliminomethyl-phenol, NT_4 (4) were synthesized using vanillin and four aromatic amines: nicotinic acid hydrazide, 2,4-dinitrophenylhydrazine, isoniazid and aniline and their anti-salmonellal activity examined and reported in this work for the first time. The synthesized compounds were characterized by Nuclear Magnetic Resonance (NMR), Fourier Transformer-Infrared (FT-IR) and melting points. The anti-salmonellal activity of the synthesized compounds was studied in comparison with their reactants (amines and vanillin) using the disc diffusion and micro dilution method using 14 isolates of *Salmonella typhi*. The activity of compounds was consistent in both assays. The compounds showed overall relatively low *in vitro* bacteriostatic activity.

Keywords

Vanillin, Anti-Salmonellal, Disc Diffusion, Micro-Dillution, Schiff Base

1. Introduction

Typhoid fever amongst other infectious diseases is still a serious public health concern in terms of severity and frequency of occurrence [1] [2]. The increased

cost of conventional anti-typhoid drugs and an increase in resistance are making them to become more unavailable to patients in Africa [2] [3]. Typhoid fever is caused by *Salmonella spp*, a common intestinal pathogen that cause food poisoning and intestinal diseases [4]. Also, there is increasing global public health concern for humans and animals antimicrobial resistance by *Salmonella species [5].* This situation has provided the impetus to the search for new antimicrobial substances from various sources including Schiff bases.

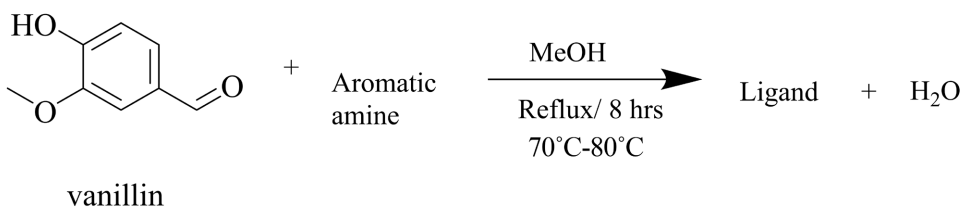
Schiff bases are compounds carrying an imine or azomethine (–C=N–) functional group prepared through a condensation reaction of primary amines and carbonyl containing compounds [6] [7] and have gained importance in present days as they are found to have a versatile pharmacophore for design and development of various bioactive lead compounds [8]. Vanillin (4-hydroxy-3-methoxybenzaldehyde) exhibits antimicrobial properties [9]. Moreover, Schiff bases derived from vanillin have been reported to have broad biological importance including antibacterial activity [10]. This provided the basis to study the antibacterial activity of Schiff bases derived from vanillin.

2. Material and Methods

The melting points of the compounds were determined on a microprocessor melting point apparatus Zenithlab. The purity of all the compounds was routinely checked by TLC on Silica gel-GF 254 (Merck) coated plates. IR spectra were recorded on a Thermo Nicolet Nexus 670-FTIR. ^{13}C NMR and ^{1}H NMR spectra were recorded on a BrukerAvance III-HD operating at 150.90 MHz and 600.13 MHz respectively. Chemical shifts are reported in δ ppm using the internal standard TMS. All the solvents and chemicals used were of analytical grade. The biological activity of the synthesized compounds was determined by the disc diffusion method.

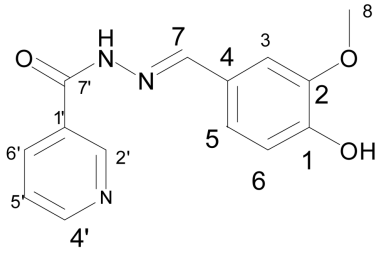
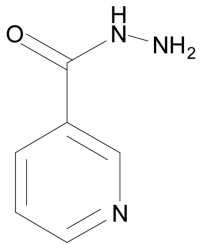
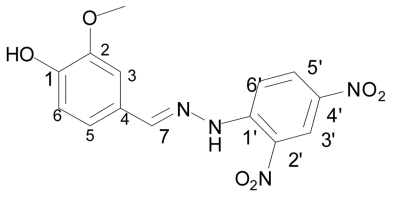
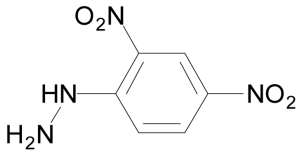
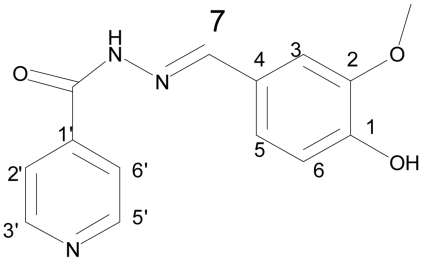
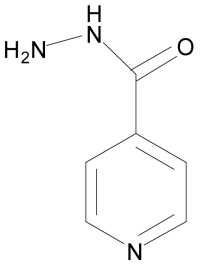
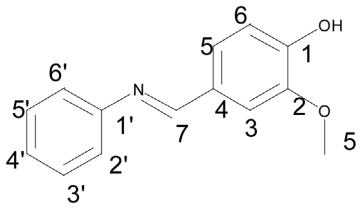
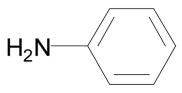
Synthesis of the Schiff base compounds

The compound (NT_1) was obtained by refluxing equimolar proportion of vanillin (0.152 g; 1.0 mmol) and nicotinic acidhydrazide (0.137 g; 1.0 mmol) in methanol (10 mL). The mixture was heated under reflux between 70˚C - 80˚C with continuous stirring for 8 hours. A pale yellow precipitate was filtered, washed with cold methanol and dried. The same procedure was used in preparing NT_2, NT_3 and NT_4 as represented in Scheme 1 using the amines shown on Table 1 below.



Scheme 1. General method of synthesis of the Schiff bases [9] [10].

Table 1. Schiff bases and aromatic amines used for their syntheses.

Compounds	Structural formula and name of amine used
 **NT_1 (1)**	 Nicotinic acid hydrazide (**5**)
 **NT_2 (2)**	 2,4-dinitrophenylhydrazine (**6**)
 **NT_3 (3)**	 isoniazid (**7**)
 **NT_4 (4)**	 aniline(**8**)

Biological screens

A total of 14 Salmonella strains were tested. Twelve (12) multidrug resistant *Salmonella* isolates from clinical specimens in health facilities in the South West Region, Cameroon, were identified by microscopy and biochemical tests using API 20E kit (Biomerieux SA, France); they were resistant to phenicol, beta-lactam, cephalosporin, fluoroquinolone antibiotics. Two (2) control strains (*S. typhimurium* ATCC 14,028 and *S. enteritidis* ATCC 13,076) were obtained from American Type Culture Collection, Manassas, USA.

The activity of the compounds was determined by the disc diffusion method at 50 µg per disc and dilutions of 1 to 512 µg/mL to determine the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) [11]. Ciprofloxacin was used as positive control antibiotic and experiments were conducted in

duplicate and twice. Activities were interpreted based on references data (CLSI, 2018) [12].

3. Results and Discussion

Four compounds (NT_1 - NT_4) were obtained by refluxing equimolar proportion of vanillin and the amine in methanol. The amines, vanillin and the synthesized derivatives were screened against resistant isolates of *Salmonella*.

3.1. Physical Properties

Physical properties such as melting point, color and physical states of synthesized compounds were recorded as shown in Table 2.

Table 2. Physical properties of the synthesized Schiff bases.

Compounds	Molecular formula	Melting point	Physical state and colour	Actual yield	Percentage yield
NT_1 (1)	$C_{14}H_{13}N_3O_3$	262˚C - 264˚C	Pale yellow powder	0.214 g	78%
NT_2 (2)	$C_{14}H_{12}N_4O_6$	240˚C - 242˚C	Red powder	0.225 g	67%
NT_3 (3)	$C_{14}H_{13}N_3O_3$	260˚C - 263˚C	Yellow solid	0.257 g	94%
NT_4 (4)	$C_{14}H_{13}NO_2$	100˚C - 103˚C	Pale yellow solids	0.259 g	57%

3.2. Spectroscopic Analysis

NMR analysis

The ^{13}C NMR spectra (150 MHz, DMSO-d_6) and ^{1}H NMR spectra (600 MHz, DMSO-d_6) are summarized in Table 3. It is worth mentioning that compounds: NT_1, NT_2, NT_3 and NT_4 have being previously synthesised [13] [14] [15] but no evaluation of their anti-salmonellal activity has been published.

Table 3. ^{13}CNMR and ^{1}HNMR data in (δ ppm) of the Schiff base compounds

Position	NT_1(1)		NT_2 (2)	NT_3 (3)	NT_4 (4)
	^{13}C shifts (ppm)	^{1}H shifts (ppm)			
1	148.3	11.80, s, OH	−11.55, s OH	−11.85, s OH	9.72, s, OH,
2	149.6	-			
3	123.5	7.29, s, 1H	7.36, 1H, s	7.28, 1H, s	7.48, 1H, s
4	114.7	-	-	-	-
5	123.9	7.06, d, 1H	7.15, d, 1H	7.07, d, 1H	7.35, d, 1H
6	108.6	6.82, d, 1H	6.85, d, 1H	6.82, d, 1H	6.86, d, 1H
7	151.8	8.29, s, 1H	8.54, s, 1H	8.31, s, 1H	8.40, s, 1H
8	55.1	3.80, s, 3H	3.82, s, 3H	3.83, s, 3H	3.81, s, 3H
NH	-	9.56, s, 1H	9.67, s, 1H	9.58, s, 1H	-
1’	129.5	-	-	-	-
2’	150.4	9.01, s	-	7.77, d, 1H	7.17, d, 1H

Continued

3’	-	-	8.83, s, 1H	8.74, d, 1H	7.30, t,1H
4’	148.0	8.72, d, 1H	-	-	7.24, t, 1H
5’	125.8	7.51, dd, 1H	8.33, 1H, d	8.74, d, 1H	7.30, t,1H
6’	136.1	8.19, d, 1H	8.05, 1H, d	7.77, d,1H	7.17, d,1H
7’	163.2	-	-	-	-

IR analysis

The characteristic IR band of the Schiff bases are shown in Table 4. The stretching vibrations of hydrogen bonded NH revealed broad bands with the maximum at about 3362 cm^{-1} in the spectra of the Schiff bases [16]. The compounds exhibit stretching vibration frequencies of imino bond formed in the range 1593 - 1668 cm^{-1}. Intensive band originating from stretching vibrations of hydrogen bonded C=O group of hydrazide moiety is at 1651 and 1650 cm^{-1} for NT_1 and NT_2 respectively in the spectra of both compounds [16] [17].

Table 4. Relevant IR bands of the Schiff bases.

Assignment	NT_1 (1)	NT_2 (2)	NT_3 (3)	NT_4 (4)
OH (cm^{-1})	3493	3274	3416	3645
NH (cm^{-1})	3274	3362	3231	-
C=O (cm^{-1})	1651	-	1650	-
C=N (cm^{-1})	1639	1606	1593	1668

Antibacterial activity

Nine (09) compounds tested at 50 µg produced zones of inhibition against a total of 14 isolates and strains with the highest zone per compound ranging from 8mm to 12 mm which indicates weak activity (Table 5); the positive control ciprofloxacin was generally highly active with almost all zones ≥ 25 mm. The lowest MIC values per compound against the 14 isolates and strains were in the range 16 to 1024 µg/mL, with compounds **3, 8** and **9** recording relatively low MICs (16 and 32 µg/mL), against just two control strains and two multidrug resistant Salmonella isolates. Compound **9** showed the lowest MIC value of 16 µg/mL on only one resistant isolate. These low MICs are comparable to the highest activity (lowest MICs) of folate antagonists (sulfamethoxazole) and fosfomycins, and the moderate activity (intermediate MICs) of most other standard antibiotics against the Enterobacteraceae family to which Salmonella belongs [12]. Only compounds **NT_3** recorded MBC of 1024 µg/mL. The activity of the compounds were consistent in both assays and compound **9** was the most active. The variation in activity is likely due to differences in resistance in the strains tested. Overall, the compounds showed relatively low bacteriostatic activity which could be improved with further structure modification. In comparison, condensation products (**1**-**4**) are less active the vanillin (**9**) while compound **NT_1, NT_2** and **NT_3** were more active than their amines **5, 6** and **7** respectively.

Table 5. Anti-salmonella activity of vanillin substituted Schiff bases.

Compound	Activity Against 14 Salmonella Isolates/Control Strains		
	Highest Zone Diameter (mm)	**MIC Range** (μg/mL)	**MBC** (μg/mL)
NT_1	11	64 - 512	Nil
NT_2	10	64 - 1024	Nil
NT_3	10.5	32 - 1024	1024
NT_4	11	256 - 1024	Nil
5*	8	1024*	Nil
6	10	128 - 512	Nil
7	10	128 - 512	Nil
8	12	32 - 512	Nil
Vanillin (**9**)	10	16 - 128	Nil
Ciprofloxacin (5 μg)	25	ND	ND

Nil: No MBC recorded in concentration range tested. *Least active compound with MIC recorded against only one isolate.

4. Conclusion

This work describes the synthesis of Schiff bases derived from vanillin. Four Schiff bases of vanillin with various aromatic amines (nicotinic acid hydrazide, 2, 4 DNPH, isoniazid and aniline) were synthesized and characterised by ^{13}C NMR, ^{1}HNMR. The anti-salmonellal activity of the Schiff bases, amines and the aldehyde (vanillin) were bacteriostatic and in general relatively low. Condensation products (**NT_1 - NT_4**) are less active the vanillin (**9**) while compound **NT_1, NT_2** and **NT_3** were more active than their amines. These results rekindle the synthesis of more analogues especially those containing F, NO_2, -OCH_3. The Schiff base analogues will be complexed with selected transition metals and later screened for biological activities.

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Conflicts of Interest

The authors declare no conflict of interest

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