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Synthesis, characterization and antimicrobial evaluation of schiff base derived from sulfonamides and vanillin

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Dana Ameen[1] Sarhang Hayyas[1*]

Abstract

Background and objective: Sulfonamides are a type of antibiotic that are used to treat several infections caused by microorganisms. Sulfonamides are bacteriostatic, meaning they prevent bacterial growth. This study aimed to produce compounds with better antimicrobial activity.

Methods: By condensation reaction, Schiff bases were synthesized from sulfonamides (sulfamethoxazole, sulfapyridine, sulfathiazole, sulfadiazine and sulfisoxazole) and vanillin to enhance antimicrobial activity of synthesized compounds. The agar dilution method was used to measuring the minimum inhibition concentration (MIC) of synthesized compounds.

Results: The synthesized compounds were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and tested for the antibacterial activity against gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria *(Escherichia coli*) and antifungal activity against (*Candida albicans*). Ciprofloxacin and Fluconazole used as standard drugs for bacteria and fungi.

Conclusion: The Schiff base was synthesized, characterized, and exhibited enhanced antimicrobial and antifungal activities.

Keywords: Sulfonamide; Schiff base; Synthesis; Vanillin; Antibacterial activity; Antifungal activity.

Introduction

Sulfonamides are a type of synthetic bacteriostatic antibiotics that are still used to treat several bacterial infections caused by microorganisms.[1] They are also known as sulfa medications, and they used as the main source for treatment of bacterial infections such as urinary tract, ear, eye infections and bronchitis.[2] Sulfonamides are compounds that containing sulfur directly attacked to benzene ring in a $-SO_2NH_2$ moiety,[3] as shown in (Figure 1).

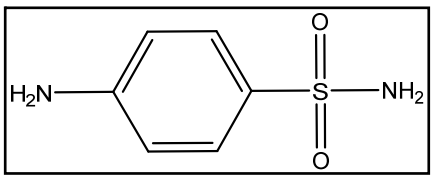

Figure 1 General structure of sulfonamide.

During the late 1930s, many different sulfonamides were developed. Most of them were found to have significant antibacterial activity against a variety of *pneumonococci* and *streptococci* bacteria. Till now there are over 5000 sulfa drugs available, but only 33 have been used in general medicine. Prontosil a red azo dye, was one of the first as sulfonamides discovered by Domagk in 1935.[4] In *vivo*, it worked against streptococcal infection, but not in vitro.

This observation was finally confirmed when it was founded that bacteria in the intestines metabolized prontosil to sulfanilamide, as the active metabolite,[5] as shown in (Scheme 1).

Sulfonamides are bacteriostatic, meaning they prevent or limit bacterial growth. sulfonamides act by inhibiting synthesis of folic acid in bacteria.[6] The mechanism of

[1] Department of Pharmaceutical Chemistry, College of Pharmacy, Hawler Medical University, Erbil, Iraq.
Correspondence: sarhanghayas@gmail.com

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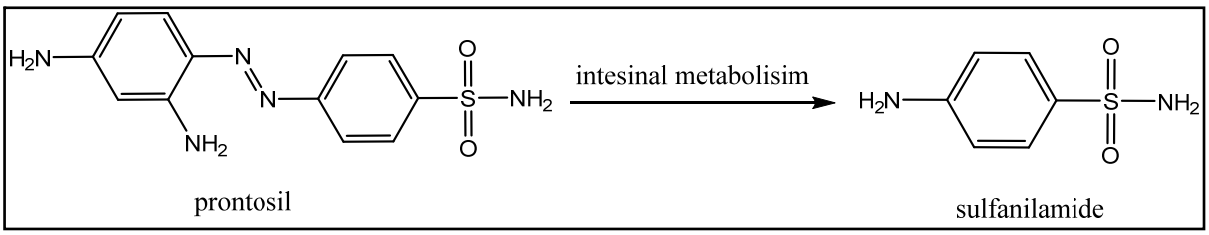
action for sulfonamides are clear, by the main chemical component that involved is para-aminobenzoic acid (PABA). Which is the essential compound for in the synthesis of dihydrofolic acid. Sulfonamides are competitive antagonists of p-aminobenzoic acid (PABA), a chemical compound required for folic acid synthesis and bacterial growth. Sulfonamides have a bacteriostatic effect when they reversibly block folic acid synthesis.[7] Sulfonamide derivatives are used as pharmacological agents having a broad range of biological activities in clinical medicine.[8]

Sulfonamides have also been shown to have antibacterial, antiviral, hypoglycemic, diuretic and anti-cancer properties. Schiff base compounds have the azomethine (imine) group (–RC=N–) are often created by condensation a primary amine with an active carbonyl molecule were prepared by Hugo Schiff in 1864.[9] The study of Schiff base coordination chemistry study has grown significantly in recent years.

The presence of a lone pair of electrons in the sp2 hybridized orbital of the nitrogen atom of the azomethine group has been shown in several studies to have significant chemical and biological importance.[10] Due to the presence of the (HC=N group), Schiff base ligands are important complexes for coordinating medicine and chemistry.[11] Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes in bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and production of molecules with unusual properties. They have anti-oxidative activity, anti-bacterial activity, anti-fungal activity and bio-inorganic activity.[12]

The main ingredient of vanilla beans is vanillin (4-hydroxy-3-methoxybenzaldehyde) as shown in (Figure 2), which is created naturally during a multi-step curing process, however 90% of vanillin currently in use is synthetically produced from lignin, eugenol. Vanillinis employed as a flavoring and aroma ingredient in the food and fragrance industries, and it is generally recognized as safe (GRAS) status. In the chemical and pharmaceutical fields, synthetic vanillin is used as an intermediary in the production of herbicides and pharmaceuticals.[13] This study aimed to synthesis series Schiff base derived from sulfonamides and vanillin. It also aimed to produce compounds with better antimicrobial activity.



Scheme 1 Conversion of prontosil to sulfanilamide.

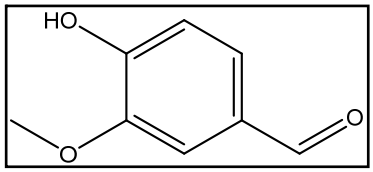


Figure 2 Chemical structure of vanillin.

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Methods

From October 2021 to April 2022, this experiment was conducted at Hawler Medical University's College of Pharmacy.

Synthesis protocol for Schiff base V1-5

0.25 gram (1mmol) of sulfonamides was dissolved in 20 ml of 100% ethanol and then added gradually to a solution of Vanillin. 0.15 gram (1 mmol) in 10 ml of absolute ethanol, mixture transferred to 100 ml round bottle flask, 2-3 drops of glacial acetic acid were added as a catalyst, and the mixture was refluxed at 83 ^{0}C for 12 hrs. The reaction was monitored using TLC. After being completed, the mixture was cooled to room temperature and then evaporated using a rotary evaporator. The solid product was recovered and purified by recrystallization from ethanol to get the required chemical as depicted in Scheme 2.

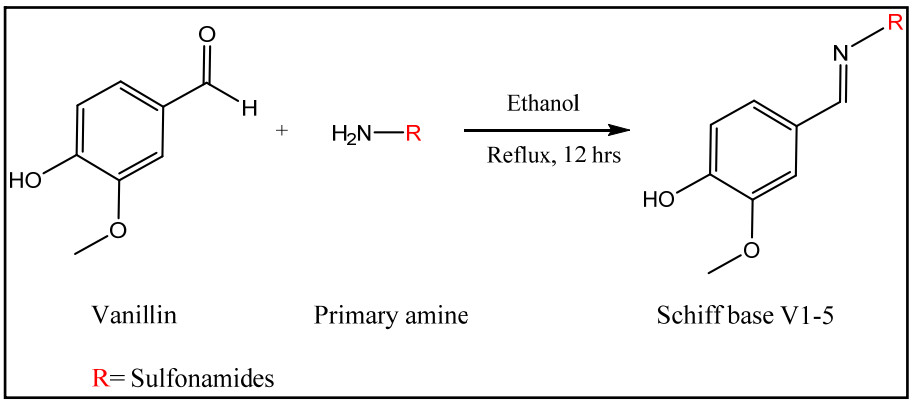
Results

The melting points of synthesized target compounds were determined and that found to be different from starting materials. And also, appearance and chemical formula of synthesized compounds different from starting materials.

Physical properties of synthesized compounds (V1-5) including melting point, appearance, percentage of yield and chemical formula are shown in (Table 1).

Table 1 Physical properties of synthesized Schiff base

Compound	Chemical formula	Yield (%)	Melting point ^{0}C	Appearance
V1	$C_{18}H_{17}N_3O_5S$	80	126-128	Yellow crystal powder
V2	$C_{19}H_{17}N_3O_4S$	85	135-137	Pale yellow powder
V3	$C_{17}H_{15}N_3O_4S_2$	82	140-142	Yellowcrystal powder
V4	$C_{18}H_{16}N_4O_4S$	77	150-152	Dark yellow powder
V5	$C_{19}H_{19}N_3O_5S$	74	145-147	Light yellow powder



Scheme 2: Synthesis of Schiff base.

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Antimicrobial study

Antimicrobial tests were conducted on synthesized compounds to assess the effectiveness of the Schiff base produced from sulfonamide. The dilution method was utilized to determine the Minimum Inhibition Concentration (MIC) of produced drugs in vitro by broth microdilution method to determination antibacterial activity with gram positive bacteria *Staphylococcus aureus* (*S. aureus*) and gram-negative bacteria *Escherichia coli* (*E. coli*) and antifungal activity with *Candida albicans* (*C. albicans*). The results were compared with Ciprofloxacin (CIP) and Fluconazole (FLU) as standard drug as shown in (Table 2).

The method was conducted by using the microtiter plate for detection the MIC against tested microbial strains. Then, various volume (µL) of different chemical materials (800, 600, 400, and 200 µg/mL), were pipetted from the stock chemical materials (1000 µg/mL) in a series of microtiter plate wells. The microtiter plate well was incubated for 24 hrs. at 37°C.

Spectroscopic study of synthesized compounds.

FT-IR spectroscopy was used to analyze Sulfisoxazole, results are shown in Figure 3, and FT-IR, 1H-NMR, and ^{13}C-NMR spectroscopy were used to analyze the synthesized molecule (V5), results are shown in Figures 4, 5, and 6.

Table 2 Results of antimicrobial activity of synthesized compounds.

	Minimum Inhibitory Concentration (µg/mL)		
Compound	***S. aureus***	***E. coli***	***C. albicans***
V1	400	400	200
V2	400	400	400
V3	200	800	800
V4	400	600	600
V5	600	200	400
CIP	50	50	-
FLU	-	-	200

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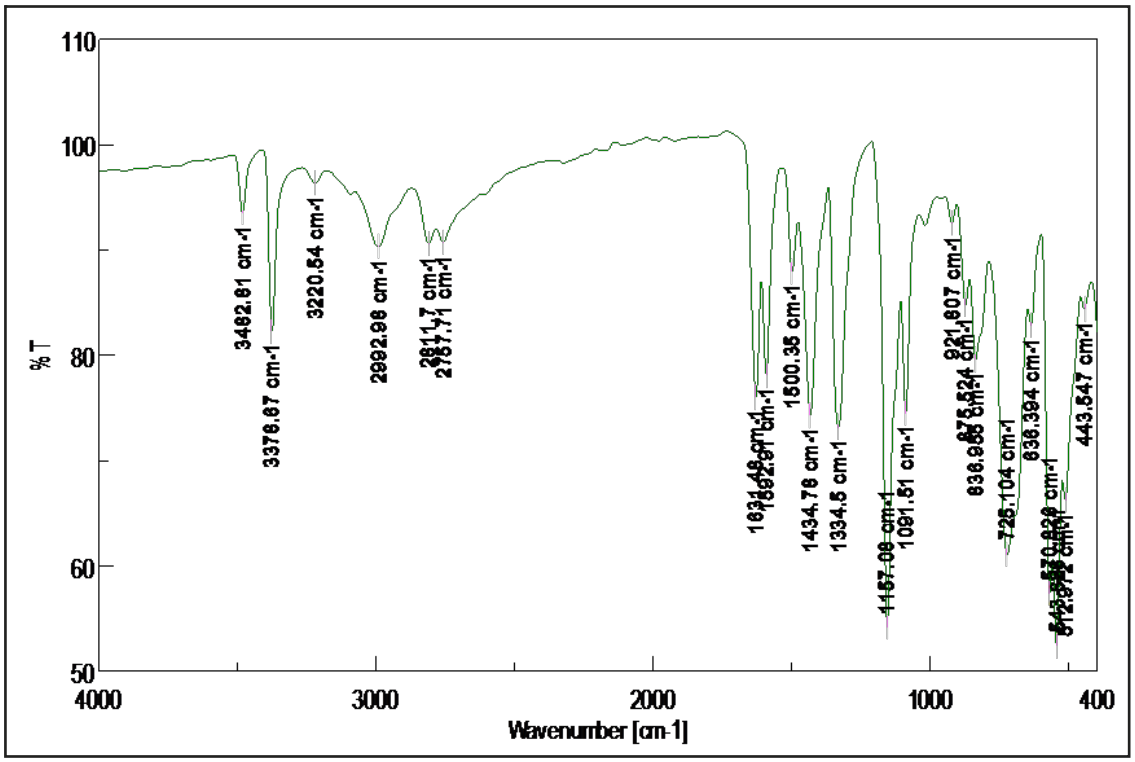

Figure 3 FTIR spectrum of Sulfisoxazole.

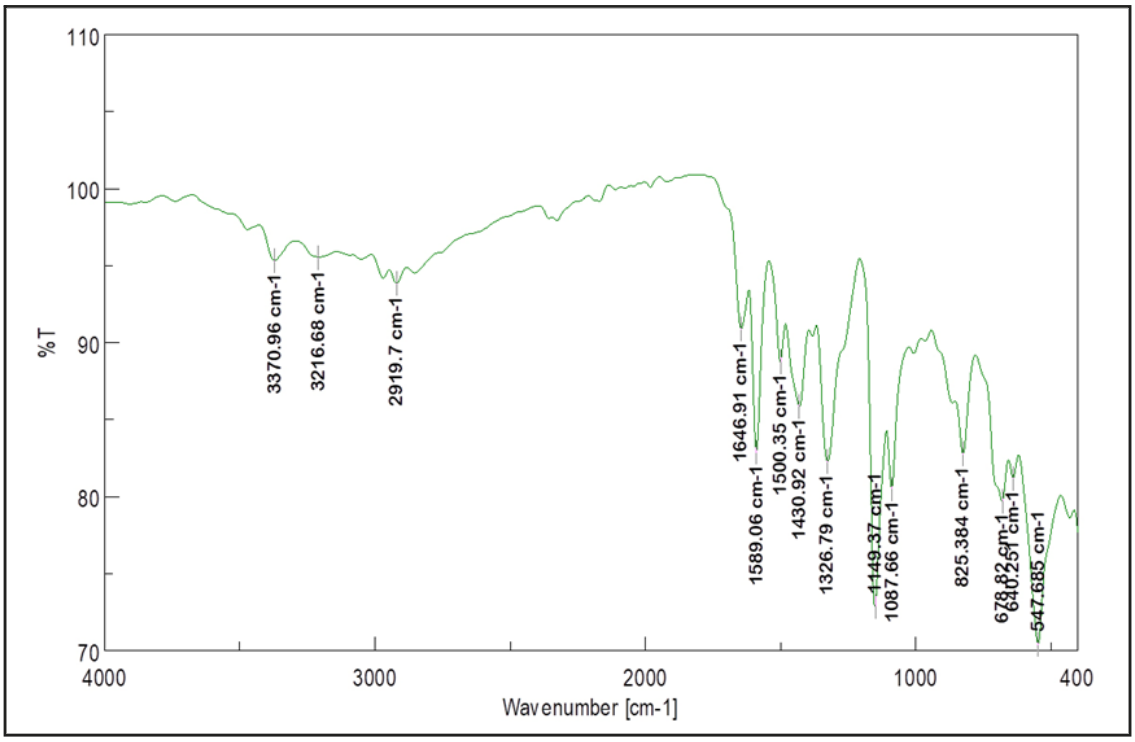

Figure 4 FTIR spectrum of V5.

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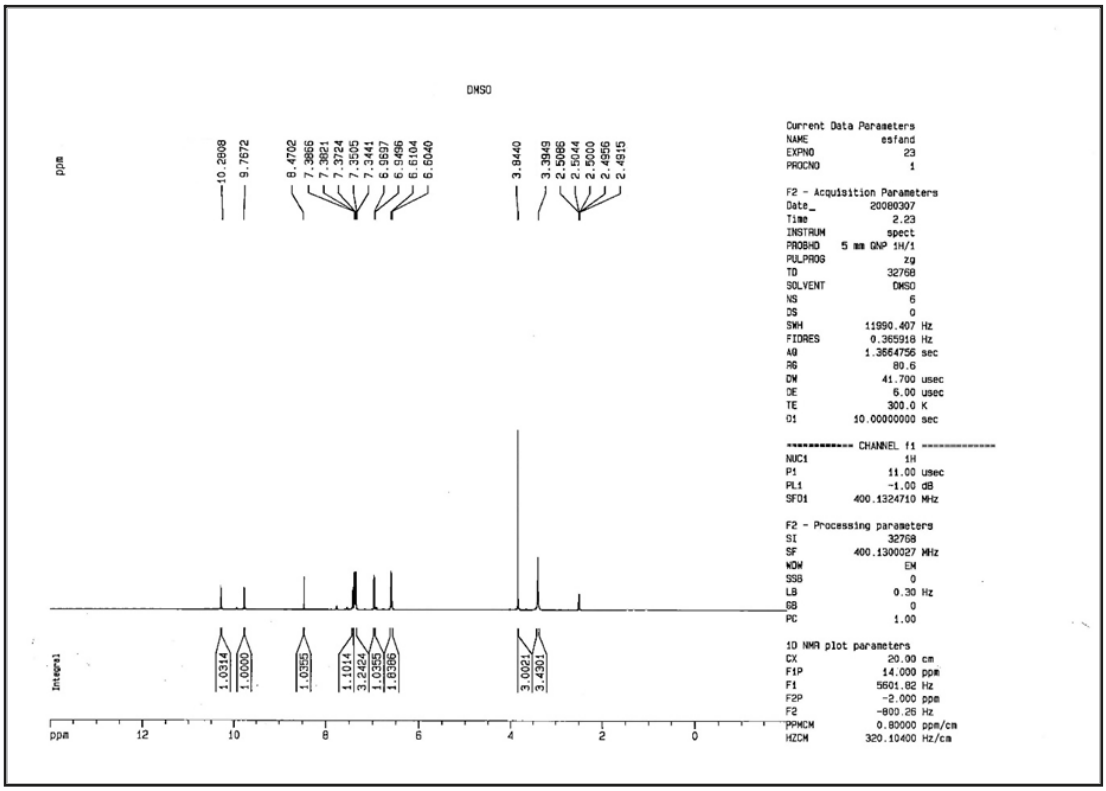

Figure 5 ^{1}H-NMR spectrum of (V5).

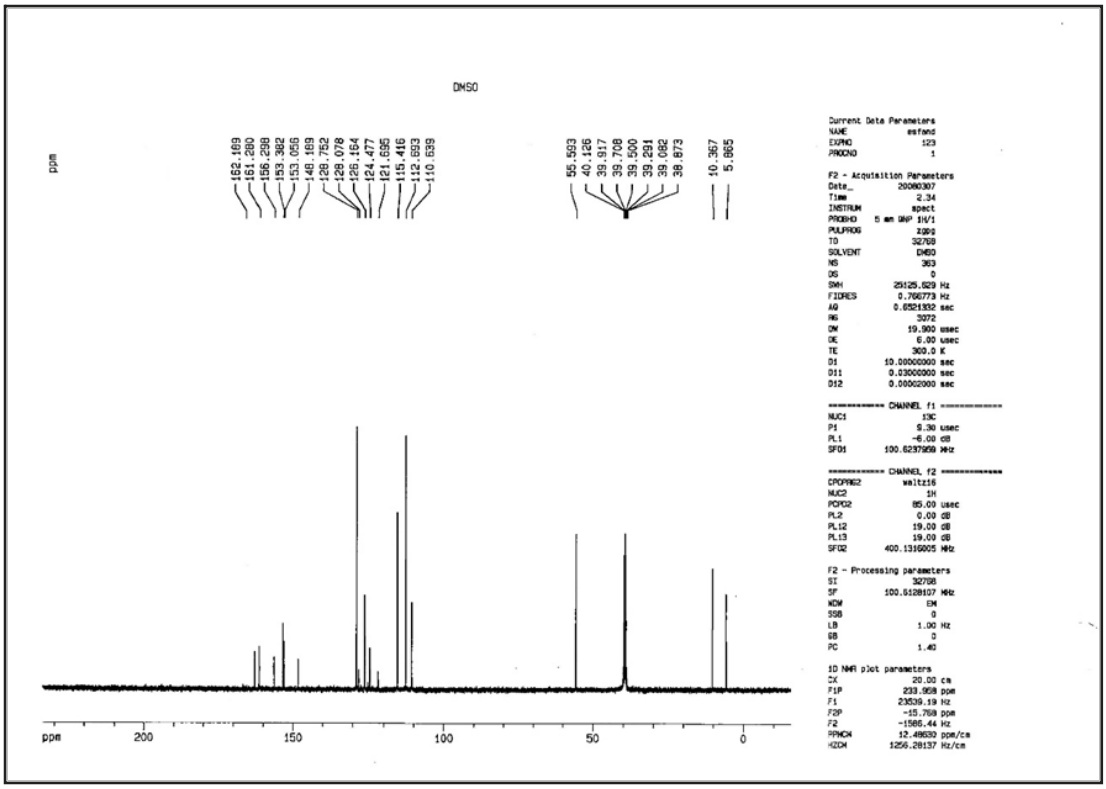

Figure 6 ^{13}C-NMR spectrum of (V5).

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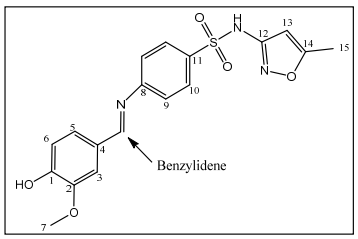
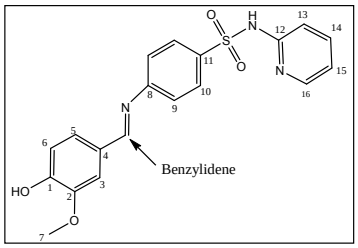
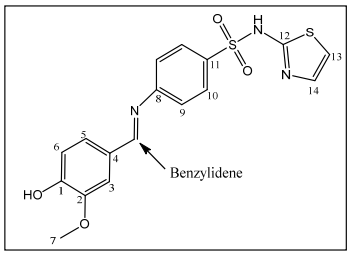
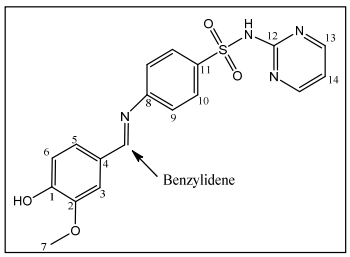
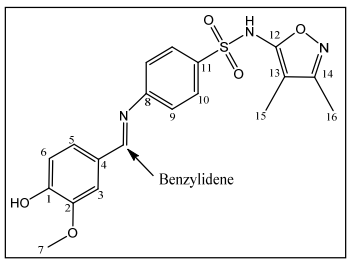
The synthesized compounds (V1-5) were analyzed using spectroscopic techniques including FT-IR, 1H-NMR, and ^{13}C-NMR. The results and interpretations are presented in Table 3.

Table 3 FT-IR, ^{1}H-NMR and ^{13}C-NMR of synthesized compounds.

Compounds	Spectral data
V1 	IR (cm^{-1} bands):1658 (C=N str), 3077 (OH str), 3382 (N-H str). ^{1}H-NMR (ppm peaks) (400 MHz, DMSO):8.42(HC=N), 9.75 (OH), 6.16-7.86 (Ar-H), 10.92 (N-H). ^{13}C-NMR (ppm peaks) (101 MHz, DMSO):163.13 (C-benzylidene),153.71 (C-1), 148.47 (C-2), 113.03(C-3), 129.27 (C4),55.99 (C-7), 151.36 (C-12), 95.70 (C-13), 170.78 (C-14), 12.35 (C-15).
V2 	IR (cm^{-1} bands):1666 (C=N str), 3259 (OH str), 3336 (N-H str). ^{1}H-NMR (ppm peaks) (400 MHz, DMSO):8.44 (HC=N), 9.75 (OH), 6.60-7.92 (Ar-H), 9.89 (N-H). ^{13}C-NMR (ppm peaks) (101 MHz, DMSO):162.51 (C-benzylidene),152.33 (C-1), 151.16 (C-2), 112.95(C-3), 127.91 (C-4), 56.01 (C-7), 153.43 (C-12), 110.92 (C-13), 140.96 (C-14), 121.61 (C-15). 148.47 (C-16).
V3 	IR (cm^{-1} bands):1670 (C=N str), 3100 (OH str), 3367 (N-H str). ^{1}H-NMR (ppm peaks) (400 MHz, DMSO):8.42 (HC=N), 9.75 (OH), 6.57-7.86 (Ar-H), 10.24 (N-H). ^{13}C-NMR (ppm peaks) (101 MHz, DMSO):163.20 (C-benzylidene),153.71 (C-1), 151.36 (C-2), 110.96(C-3), 129.27 (C-4), 55.99 (C-7), 170.31 (C-12), 113.03 (C-13), 135.96(C-14).
V4 	IR (cm^{-1} bands):1650 (C=N str), 3135 (OH str), 3355 (N-H str). ^{1}H-NMR (ppm peaks) (400 MHz, DMSO):7.93 (HC=N), 9.75 (OH), 6.54-7.61 (Ar-H), 11.24 (N-H). ^{13}C-NMR (ppm peaks) (101 MHz, DMSO):163.13 (C-benzylidene),153.71 (C-1), 151.36 (C-2), 110.96 (C-3), 129.27 (C-4), 55.99 (C-7), 170.31 (C-12), 158.39 (C-13), 113.03(C-14).
V5 	IR (cm^{-1} bands):1646 (C=N str), 3200 (OH str), 3370 (N-H str). ^{1}H-NMR (ppm peaks) (400 MHz, DMSO):8.47 (HC=N), 9.76 (OH), 6.60-7.38 (Ar-H), 10.28 (N-H). ^{13}C-NMR (ppm peaks) (101 MHz, DMSO):162.18 (C-benzylidene),153.05 (C-1), 148.18 (C-2), 112.69 (C-3), 128.07 (C-4), 52.59 (C-7), 156.29 (C-12), 110.63 (C-13), 161.28 (C-14), 5.86 (C-15). 10.36 (C-16).

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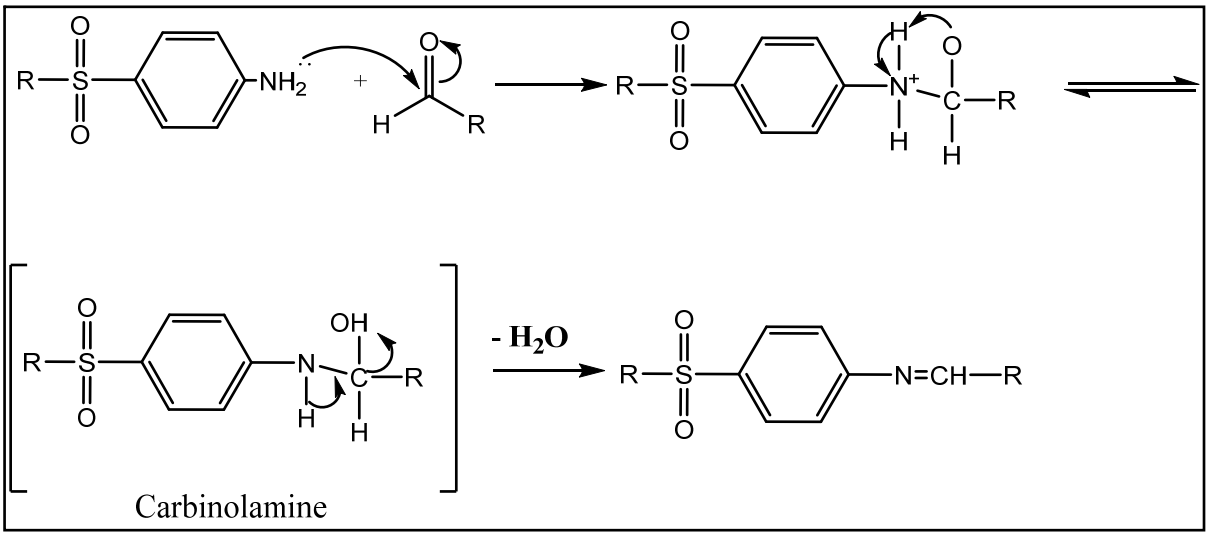
Discussion

The synthesis of Schiff base from aldehyde and amine is a reversible chemical reaction that takes place under acid or base catalysis. Schiff base compounds are often produced by two types of chemical reactions: addition and elimination. Most Schiff bases are produced under slightly acidic conditions.[14] The production of the Schiff base occurs through the nucleophilic addition to the carbonyl group of the substrate. The nucleophile in this case was an amine. The amine molecule underwent a reaction with the aldehyde molecule to produce an unstable chemical called carbinolamine. The carbinolamine loses water through either acid or base catalyzed routes to produce Schiff base.[15] As seen in (Scheme 3).

The infrared spectra of synthesized compound (V1) showing disappearance of absorption bands in (3463 cm^{-1}) and (3293 cm^{-1}) in IR spectrum of (V1), while which presented in IR spectrum of (sulfamethoxazole) due to NH_2 stretching vibration, appearance of absorption band in (1656 cm^{-1}) in IR spectrum of (V1) due to C=N stretching vibration, The ^{1}H-NMR of compound (V1) shows multiple signals at around (6.16-7.86 ppm) due to H-aromatic, a singlet signal at (8.42 ppm) for proton of imine group, singlet signal at (9.75 ppm) for OH group, The ^{13}C-NMR of compound (V1) shows multiple signals in different region such as 163.13 (C-benzylidene), 153.71 (C-1), 148.47 (C-2), 113.03(C-3), 129.27 (C4),55.99 (C-7), 151.36 (C-12), 95.70 (C-13), 170.78 (C-14), 12.35 (C-15). The infrared spectra of compound (V2) showing disappearance of absorption bands in (3471 cm^{-1}) and (3293 cm^{-1}) in IR spectrum of (V2), while which presented in IR spectrum of (sulfapyridine) due to NH_2 stretching vibration, appearance of absorption band in (1666 cm^{-1}) in IR spectrum of (V2) due to C=N stretching vibration, The ^{1}H-NMR of compound (V2) shows multiple signals at around (6.60-7.92 ppm) due to H-aromatic, a singlet signal at (8.44 ppm) for proton of imine group, singlet signal at (9.75 ppm) for OH group, The ^{13}C-NMR of compound (V2) shows multiple signals in different region such as 162.51 (C-benzylidene), 152.33 (C-1), 151.16 (C-2), 112.95(C-3), 127.91 (C-4), 56.01 (C-7), 153.43 (C-12), 110.92 (C-13), 140.96 (C-14), 121.61 (C-15). 148.47 (C-16). The infrared spectra of compound (V3) showing disappearance of absorption bands in (3274 cm^{-1}) and (3089 cm^{-1}) in IR spectrum of (V3), while which presented in IR spectrum of (sulfathiazole)



Scheme 3 Mechanism of Schiff base synthesis.

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due to NH_2 stretching vibration, appearance of absorption band in (1670 cm^{-1}) in IR spectrum of (V3) due to C=N stretching vibration, The ^{1}H-NMR of compound (V3) shows multiple signals at around (6.57-7.86 ppm) due to H-aromatic, a singlet signal at (8.42 ppm) for proton of imine group, singlet signal at (9.75 ppm) for OH group, The ^{13}C-NMR of compound (V3) shows multiple signals in different region such as 163.20 (C-benzylidene), 153.71 (C-1), 151.36 (C-2), 110.96 (C-3), 129.27 (C-4), 55.99 (C-7), 170.31 (C-12), 113.03 (C-13), 135.96 (C-14). The infrared spectra of compound (V4) showing disappearance of absorption bands in (3417 cm^{-1}) and (3255 cm^{-1}) in IR spectrum of (V4), while which presented in IR spectrum of (sulfadiazine) due to NH_2 stretching vibration, appearance of absorption band in (1650 cm^{-1}) in IR spectrum of (V4) due to C=N stretching vibration, The ^{1}H-NMR of compound (V4) shows multiple signals at around (6.54-7.61ppm) due to H-aromatic, a singlet signal at (7.93 ppm) for proton of imine group, singlet signal at (9.75 ppm) for OH group, The ^{13}C-NMR of compound (V4) shows multiple signals in different region such as 163.13 (C-benzylidene), 153.71 (C-1), 151.36 (C-2), 110.96 (C-3), 129.27 (C-4), 55.99 (C-7), 170.31 (C-12), 158.39 (C-13), 113.03 (C-14). The infrared spectra of compound (V5) showing disappearance of absorption bands in (3482 cm^{-1}) and (3220 cm^{-1}) in IR spectrum of (V5), while which presented in IR spectrum of (sulfisoxazole) due to NH_2 stretching vibration, appearance of absorption band in (1646 cm^{-1}) in IR spectrum of (V5) due to C=N stretching vibration, The ^{1}H-NMR of compound (V5) shows multiple signals at around (6.60-7.38 ppm) due to H-aromatic, a singlet signal at (8.47 ppm) for proton of imine group, singlet signal at (9.76 ppm) for OH group, The ^{13}C-NMR of compound (V5) shows multiple signals in different region such as 162.18 (C-benzylidene), 153.05 (C-1), 148.18 (C-2), 112.69 (C-3), 128.07 (C-4), 52.59 (C-7), 156.29 (C-12), 110.63 (C-13), 161.28 (C-14), 5.86 (C-15). 10.36 (C-16). In vitro antibacterial activity synthesized compounds (V1-5) were tested against gram positive bacteria *S. aureus*, gram negative bacteria *E. coli* and antifungal activity with *C. albicans* by using broth micro dilution method. Minimum Inhibition Concentration of synthesized compounds were compared to Ciprofloxacin and Fluconazole as standard drugs as shown in (Table 2).

Compound V3 was found displayed greater antibacterial activity (MIC 200 µg/mL) against *S. aureus*, Compound V5 was found displayed greater antibacterial activity (MIC 200 µg/mL) against *E. coli* and Compound V1 was found displayed greater antibacterial activity (MIC 200 µg/mL) against *C. albicans*.

Conclusion

The study found that synthetic chemicals, specifically Schiff base formed from sulfonamides and vanillin, were successfully obtained based on the results presented. The structure of synthesized compounds is determined and verified using infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR). The produced chemicals are evaluated for their effectiveness against various bacteria and fungus species. V3 is particularly effective against *Staphylococcus aureus*, V5 is highly effective against *Escherichia coli*, and V1 is highly effective against *Candida albicans*.

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Jabbir AH. Synthesis of some sulfa drug derivatives as antibacterial agents: Ministry of Higher Education; 2016. DOI: 10.20546/ijcmas.2016.505.006.
2. Mok BLJ. The synthesis of functionalised sulfonamides: University of London, University College London (United Kingdom); 2008.

3. Gomathi V, Ramaswamy S. Antimicrobial Screening of Novel Schiff Bases From Sulfa Drugs. Int J Pharm Pharm Sci 2014; 6(1):487–91.
4. Mahdi MF, Al-Smaism RF, Al-Khaliq Z. Synthesis, characterization and antibacterial activity of new series of sulfamethoxazole derivatives. World J Pharm Pharmaceut Sci 2015; 4(10):284–93.
5. Ali AT, Mosa MN, Alshaheen ZG, Muhammad-Ali MA. Synthesis, characterization and antibacterial evaluation of oxoazetidin? benzene sulfonamide derivatives as a hybrid antimicrobial agents. Syst Rev Pharm 2020; 11(2):487–94. DOI: 10.5530/srp.2019.2.04.
6. Raauf AM. Synthesis, Characterization and Antibacterial Activity of New Derivatives of Imidazolidine having Sulfamethoxazole Moiety. AJPS 2019; 19(4):217–38. DOI:10.32947/ajps.19.04.0433.
7. Tačić A, Nikolić V, Nikolić L, Savić I. Antimicrobial sulfonamide drugs. Advanced Technologies 2017; 6(1):58–71. DOI:10.5937/savteh1701058T.
8. Yousef F, Mansour O, Herbali J. Sulfonamides: Historical discovery development (structure-activity relationship notes). vitro In-vivo In-silico Journal 2018; 1(1):1–15.
9. Ebrahimi H, Hadi J, Al-Ansari H. A new series of Schiff bases derived from sulfa drugs and indole-3-carboxaldehyde: Synthesis, characterization, spectral and DFT computational studies. J Mol Struct 2013; 1039:37–45. DOI: 10.1016/j.molstruc.2013.01.063.
10. Ashraf MA, Mahmood K, Wajid A, Maah MJ, Yusoff I. Synthesis, characterization and biological activity of Schiff bases. IPCBEE 2011; 10(1):185.
11. Ahmed SM, Salih KM, Ahmad HO, Jawhar ZH, Hamad DH. Synthesis, spectroscopic characterization and antibacterial activity of new series of Schiff base derived from 4-aminoantipyrine and 2-amino benzimidazole. Zanco J Med Sci 2019; 23(2):206–16. DOI: 10.15218/zjms.2019.026.
12. Abdulghani AJ, Abbas NM. synthesis characterization and biological Activity study of New Schiff and mannich bases and some metal complexes derived from isatin and dithiooxamide. Bioinorganic chemistry and applications 2011; 2011. DOI: 10.1155/2011/706262.
13. Walton NJ, Mayer MJ, Narbad A. Vanillin. Phytochemistry 2003; 63(5):505–15. DOI: 10.1016/S0031-9422(03)00149-3.
14. Solankee A, Thakor I. Synthesis of pyrazolines, isoxazolines and aminopyrimidines as biological potent agents 2006. DOI: 10.1002/chin.200622168.
15. Xavier A, Srividhya N. Synthesis and study of Schiff base ligands. IOSR-JAC 2014; 7(11):06–15.