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Antimicrobial Activity and Molecular Modeling Study of Schiff Base Derived from Sulfamerazine

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Abstract

Condensation of 4-amino-*N*-(4-methylpyrimidin-2-yl)benzene sulfonamide (Sulphamerazine Drug) with 1,3-Benzodioxole-5-carbaldehyde (Piperonal)yielded Schiff base derivative in good yield. Spectroscopic characterization such as, IR and NMR of synthesized compound have been obtained by using nuclear magnetic resonance 600 MHz. The synthesized compound was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus and klebsella pneumonia* and fungicidal activity against *Candida albicans, Candida trobicalis, Candida krusi, Aspergillus fumigatus* and *Aspergillus niger*. A new compound exhibited potent antibacterial and antifungal activity. Molecular modeling studies were performed, showing the hydrogen bindings and hydrophobic interactions.

Keywords**:** *Sulphamerazine, Piperonal, Schiff-base, Microbial activity, Molecular modeling.*

Introduction

New sources of antimicrobial agents is need to be discovered due to the presence and constant development of resistant micro-organisms, the emergence of new infections diseases and the toxicity concerns of some of the currently used antimicrobial treatments[1].

The random use of traditional antibiotics and synthetic antimicrobial drugs has resulted in the emergence of resistance microbes such as Methicillin Resistance *Staphylococcus aureus* (MRSA). Vancomycin resistance enterococci and multidrug resistance strain of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which have come to be a cause of concern for scientists to find a different approach for treating such infection. In a high percentage of asian countries 90-95% of *S.aureus* are penicillin resistance and 75% are methicillin resistance[2,3]. Sulfa drugs, developed in the 1930s, were the first medications effective against bacterial disease and infection. They seemed as the first "miracle drugs" at a time when death from bacterial infections such as pneumonia and blood poisoning were unrestricted[4]. Moreover,sulfonamide derivatives have received considerable interest from researchers, and many sulfonamide derivatives have been prepared for their importance in the pharmacological and biological fields. Several Schiff-base compounds derived from sulfonamide have been prepared for use in many biological applications[5,6].

Schiff bases are versatile C=N (Imine) containing compounds possessing broad spectrum of biological activity. Schiff base are the compound containing azomethine group (-HC=N-). They are condensation products of ketones (or) aldehydes (aldehyde and ketones) with primary amines and were first reported by Hugo Schiff in 1864[7]. R. C. Maurya *etal* were prepared some Schiff base derived from salicylaldehyde and the sulfa drug sulfamerazine, [N-(salicylidene)-sulfamerazine][8].

Schiff base derived from o-vanillin and Sulfamerazine, β – lactam and Zn(II) , Cu(I) complexes have been Synthesized and showed highly antimicrobial activity toward mentioned some bacteria[9].

The aim of present work is to synthesis of new Schiff base derived from sulfamerazine drug and piperonal (Scheme1) and study of their antimicrobial activity and molecular modeling.

Materials and Method

Physical measurements

The IR spectra were recorded in the range of 4000-200 cm^{-1} on a Pye-Unicam SP3-300 spectrometer using KBr discs at Department of Chemistry, College of Education for pure Sciences, University of Basrah. One dimensional ^{1}H, ^{13}C NMR and two dimensional HSQC and HMBC- NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz University, Germany. Melting points were measured by a Philip Harris melting point apparatus at College of Veterinary medicine, University of Basrah.

b) Synthesis

Synthesis of Schiff-base

3.78 mmol (1.0 g) of sulfamerazine in 25 ml ethanol was added to 3.73 mmol (0.567 g) of hot ethanolic solution of piperonal, three drops of glacial acetic acid was added and resulting solution was refluxed for 3h, and then lift overnight in refrigerator, the solid product obtained was filtered and washed with acetone and the final product was recrystallized by using chloroform: ethanol 8:2 to yield yellow crystals of new Schiff base.

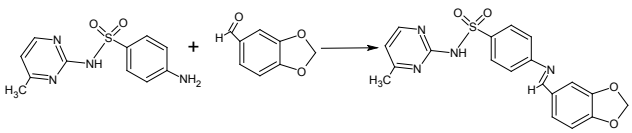
c) Antimicrobial activity

The novel synthesized compounds have been tested *in vitro* for their antibacterial activity against: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*. Additionally, the particular compounds were tested for antifungal activity against *Candida krusei*,*Candida tropicalis*, *Candida albicans*, *Aspergillus niger* and *Aspergillus fumigatus* using the paper disc-agar diffusion technique as recommended by the Clinical and Laboratory Standards Institute[10]. The test compounds were dissolved in DMSO solvent and the recommended concentrations were (50, 100 and 200µg/mL), each concentration have been used in the disc-agar diffusion technique. Ampicillin and Nystatin were used as standard controller for bacteria and fungi, respectively. Petri plates containing 20mL of Mueller Hinton agar were used as a culture medium for antibacterial activity. On the other hand the *Candida krusei*, *Candida tropicalis*, *Candida albicans*, *Aspergillus niger* and *Aspergillus fumigatus* were cultivated in Sabouraud dextrose agar as a culture medium for antifungal activity. Sterile Whatman no. 1 filter paper disks (6mm in diameter) were impregnated with the solution of DMSO and placed on the Petri plates. Additionally, another paper disk was impregnated with dimethylsulfoxide (DMSO) and used as negative control. The plates were incubated for 24 h at 37°C in the case of bacteria and 72 h. at 27°C for fungi and yeasts. The inhibition zone diameters were measured in millimeters.

Resuls

Chemistry

The present work, a new Schiff base has been synthesized. The reaction of 4-amino-N-(4-methylpyrimidin-2-yl)benzene sulfonamide (sulfamerazine drug) with 1,3-Benzodioxole-5-carbaldehyde (Piperonal) at 1:1 ratio to produce the Schiff base derivative, Scheme 1, in good yield.



Scheme 1: Preperation of Schiff base derived from sulfamerazine

Yield; 83%, M.P.= 204-205 ^{0}C. FT-IR (KBr,ν, cm^{-1}): 3400 (NH), 3068,3024(CH-aromatic), 2929,2858(CH-aliphatic), 1639-1537(C=C, C=N). ^{1}H NMR (600 MHz,DMSO-*d6*, δ, ppm): 11.13 (s,1H, NH), 8.31 (s,1H, CH=N), 7.65-5.98 (m,9H, Ar-H), 5.98(s, 2H, CH_2-O), 2.31(s, 3H, CH_3). ^{13}C NMR (DMSO-*d6*); 23.8(C-CH_3), 111.7(CH_2-O), 112.5-157(C-Ar), 158.1(C-CH=N), 168.4(C-S).

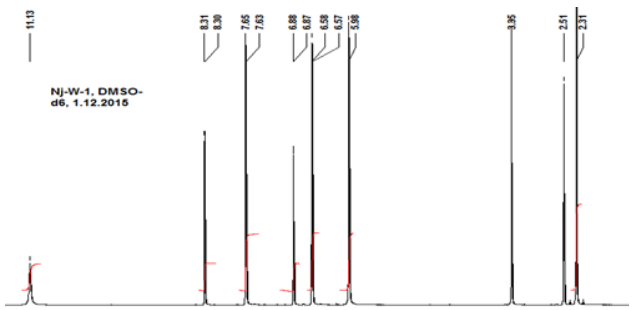


Fig. 1: ^{1}H NMR of synthesized compound

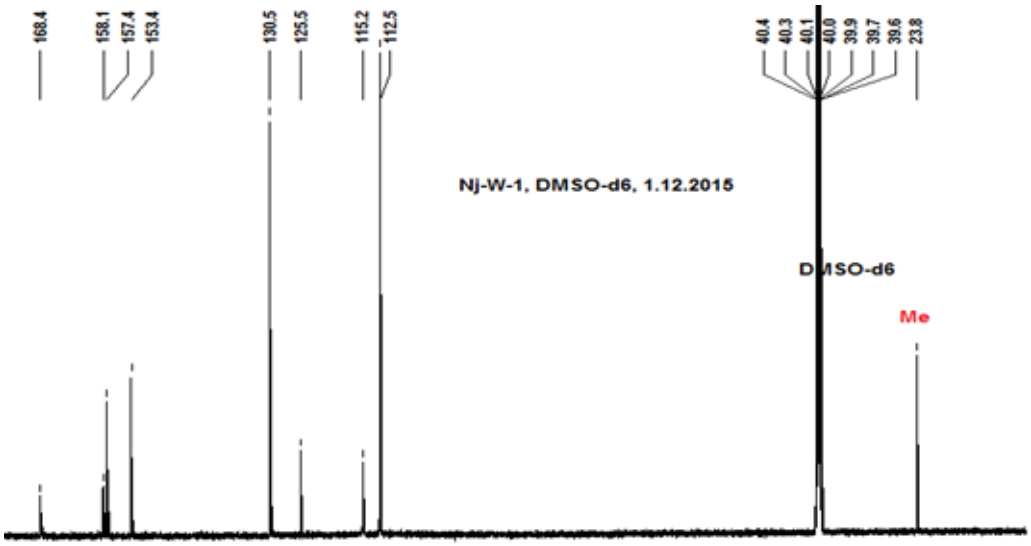


Fig. 2: ^{13}C NMR of synthesized compound

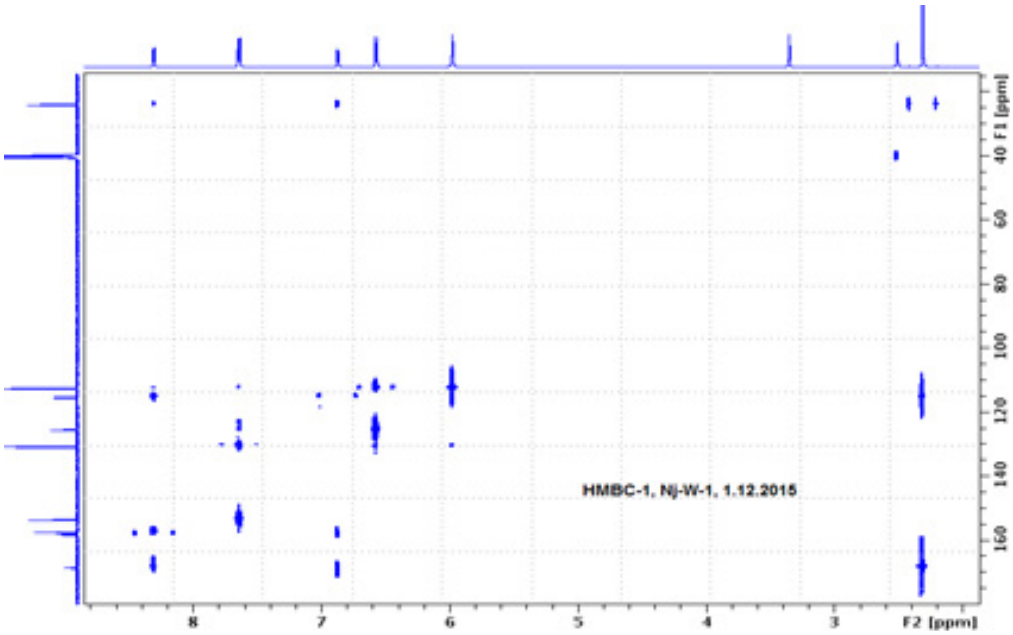


Fig. 3: HMBC- NMR of synthesized compound

Antimicrobial activity

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents[11]. In the present work the studied compound are evaluated *in vitro* for their antibacterial and antifungal activities by using paper disc-agar diffusion technique[10] with the microorganisms as seen in Table 1. Antibiotic drug ampicillin and Nystatin were used as control for bacteria and fungi, respectively.

Table 1: The antibacterial and antifungal activity of the studied compounds(inhibition zone mm)

Bacteria and fungi types	Zone inhibition of antibiotic disc (control)(mm)		Zone inhibition of antimicrobial sensitivity test of compounds (mm)					
		Compound 1(S) µg/ml			Compound 2 µg/ml			
	Ampicillin 25µg/ml	Nystatin 30µg/ml	200	100	50	200	100	50
Staphylococcus aureus	22	-	18	15	12	16	12	10
Escherichia coli	20	-	15	12	12	11	10	10
Klebsiella pneumonia	25	-	14	12	10	10	8	6
Bacillus cereus	30	-	0	0	0	0	0	0
Candida krusei	-	11	30	25	20	21	16	15
Candida tropicalis	-	-	15	12	10	18	16	12
***Candida* albicans**	-	-	18	15	12	18	15	12
Aspergillus niger	-	11	15	14	9	20	17	14
Aspergillus fumigatus	-	10	12	10	6	15	15	12

Molecular modeling analysis

The molecular docking was performed using SYBYL-X 1.1and the docking results were shown by PyMOL[12]. Our molecular docking analysis of the new analogue based on the modeling study which was performed to understand the binding mode of these analogues with UDP-N-acetylglucosamine 1-carboxyvinyl transferase of *E. coli*[13] binding pocket (PDB code:1ahg[14]. Compound **2** has been selected for the docking modelling study, since its binding energy score

-9.8, with indicating a selectivity of 4-((benzo[*d*][1,3] dioxo-5-ylmethylene)amino)-N-(5-methylpyrimidin-2-yl)benzenesulfonamide in binding to the enzyme pocket (Figure 4) via its and its azoandbenzo[*d*][1,3]dioxo groups. As shown in Figure 4, the aromatic ring of new compound was fitted into an aromatic rich sub-pocket surrounded by the aromatic side chains of Phe328 showing an hydrophobic interaction via the pi stacking, in addition to two hydrogen bonding were observed. The azo-sulfamethazine backbone was located in the middle of the binding pocket, anchoring the nitrogen atom of the azo group in a favourable position for hydrogen bonding with the NH_2 of the amino acid of *E. coli* Asn23, whereas the other hydrogen bond was assigned between the oxygen atom of benzo[*d*][1,3]dioxo group and the NH_2 group of Asn305. Overall, the combination of hydrophobic interaction and pi stacking appears to govern the binding of new compound with amino acids of transferase of *E. coli*. Furthermore, the amino acids of the enzyme: Tyr399 and Ala374 have surrounded the sulfamerazine backbone.

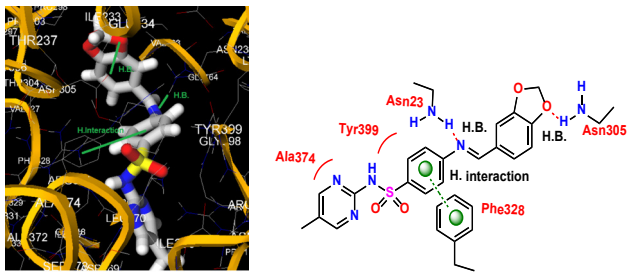


Figure 4: Docked conformation of synthesized compound showing two hydrogen bonds: NH_2 group of Asn305 with oxygen atom of benzo[*d*][1,3]dioxomoiety, and Asn23 with nitrogen atom of the azo group.

It also exhibits also hydrophobic interaction between phenyl ring of sulfamethazine residue and Phe328 of UDP-GlcNAcenolpyruvyltransf eraseenzyme residuUDP-N-acetylglucosamine 1-carboxyvinyltransferase of *E. coli* (PDB: 1A2NMurA (UDP-GlcNAcenolpyruvyltransferase), the first enzyme in bacterial peptidoglycan biosynthesis, catalyzes the enolpyruvyl transfer from phosphoenolpyruvate (PEP) to the 3'-OH of UDP-GlcNAc by an addition-elimination mechanism that proceeds through a tetrahedral ketal

intermediate. The crystal structure of the Cys115-to-Ala is shown in Fig. 5.

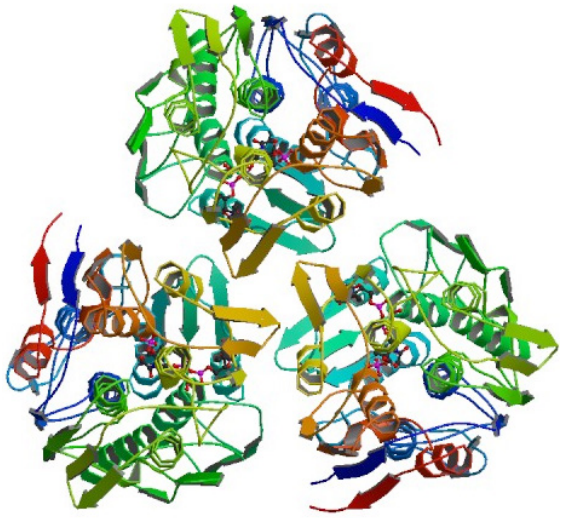


Fig. 5: UDP-N-acetylglucosamine 1-carboxyvinyltransferase of *E. coli*

Discussion

The search of novel antimicrobial agents still continues as the available antibiotics are not active against multi drug resistant pathogenic strains. In current study the Schiff base derived from sulfamerazine is synthesized and their chemical structure was confirmed by IR and NMR spectral analysis. The IR spectra confirm the presence of the azomethine group (-CH=N) stretching with a sharp region around 1540 cm^{-1}. ^{1}H NMR spectra of synthesized compound shows signal due to azomethine proton(CH=N) at 8.31ppm. ^{1}H NMR spectra of synthesized compound show a singlet at 11.13 ppm due to NH. The region at 7.65- 5.98 ppm due to aromatic protons. ^{1}H NMR spectra of synthesize compound shows singlet's at 3.35 and 2.31ppm respectively, due to aliphatic protons, Fig. 1.

The ^{13}C NMR spectrum of synthesized compound was measured in DMSO-d6. ^{13}C NMR spectra gave further support to the formation of new compound. The spectra revealed the presence of CH=N group around 158.1ppm. The signals around 157.4-112.5 ppm due to C-aromatic groups. The signal at 168.4 ppm due to C-SO_2 group, Figure 2. The two dimensional ^{1}H, ^{13}C-HMBC NMR spectra are support the chemical structure of synthesized compound, Figure 3.

The antibacterial activity of the synthesized compounds were tested against two Gram positive bacteria (*Staphylococcus aureus, Bacillus cereus,* and two Gram negative bacteria (*Klebsiella pneumonia, Escherichia coli*) at a concentration of 50, 100 and 200µg/mL using DMSO as a solvent, which have no effect on the growth of microbes[15]. According to the results on Table (1) the antimicrobial activity of the tested compounds were showed a slight to intermediate activity against *Klebsiella pneumonia* and *Staphylococcus aureus* but show no activity against *Escherichia coli* and *Bacillus cereus.* On the other hand, the synthesized compounds were showed a good activity against all the fungi species. However, the compounds had the highest effect against *Candida krusei* and *Aspergillus niger* and intermediate activity against *Candida tropicalis, Candida albicans* and *Aspergillus fumigatus.* The difference of susceptibility of the tested microorganisms could be attributed to their major properties of microorganisms that are related to the permeability of their cell surface to the antibiotics and drugs.

Conclusion

In conclusion the studied compound could be a probable source to obtain effective against multi-drug resistant strains of microorganisms. Conversely, it is necessary to calculate the MIC index (MBC/MIC) and to determine the toxicity and the side effects of the studied compounds.

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Disclosure statement

Conflict of Interests: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical Clearance: All ethical guidelines have been adhered according to committee on the ethics of dealing with laboratory animals.

Source of Funding: Self

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