

SYNTHESIS AND EVOLUTION OF SULFAMETHOXAZOLE DERIVATIVE AND ASSAY ANTIBACTERIAL ACTIVITY

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ABSTRACT

Condensation of Sulfamethoxazole with 3,4-dihydroxy benzaldehyde yielded Schiff base derivative in good yield. Characterization of synthesized compound detected by Infra-red (IR) and ¹HNMR spectroscopy via nuclear magnetic resonance 600 MHz. The synthesized compound was screened for antibacterial activity against gram positive and gram negative bacteria. A new compound exhibited potent antibacterial activity against *Klebsiella pneumonia* compared to Vancomycin drug, which showed no activity against this bacterium. The synthesized compound exhibited moderate activity against *Escherichia coli* and *Staphylococcus aureus*.

INTRODUCTION

Sulfamethoxazole (SMZ) is a one of the sulfonamide class of antibiotic, it has the chemical name 4-Amino-N-(5-methyl-3-isoxazolyl)-benzene and molecular formulation (C₁₀H₁₁N₃O₃S), with m. wt. about 253.279 g.mol⁻¹. The first starting uses of sulpha as a drug was by Domagk in 1932, since that sulfanilamides are known as a suitable bacteriostatic medicines with gram positive and gram negative bacteria (1,2).

Microbial study to SMZ clarify that it blocks germ generate of dihydrofolic acid through vying with para aminobenzoic acid in the result sulfamethoxazole prohibit a step in the biosynthesis of DNA and proteins fundamental to the bacteria, A further technique of SMZ work via deny cross-membrane transfer of glutamic acids as a primary ingredient of folic acid formation (3).

At the last years (SMZ) uses has been restricted due to expansion of resistance occurring in accordance with mistreatment in human and animals (4), So the modern research pointing on constringe the growth of drug- reluctant bacteria and keep the efficiency of sulfamethoxazole drug.

There are a lot of modified sulfonamides have been brought and have a structural change on the antibacterial performance (5-7). Characteristic structure of sulfonamides sorted it as a little molecules that there is no considerable variance could executed without changing the basic nucleus. So in our research we designed a new sulfamethaxazole derivative from Schiff base to get better antimicrobial activity.

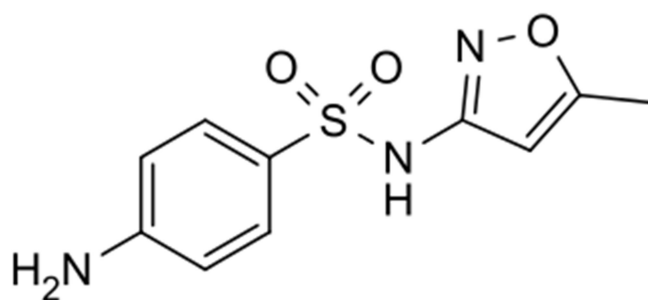


Figure 1: Chemical structure of sulfamethaxazole drug

MATERIALS AND METHODS

Chemical analysis:

FTIR, ¹H-NMR analysis used to confirm the chemical structure of the newly synthesized compound. The characteristic Infrared spectra (IR) band for this compound display at rang 4000-200 cm⁻¹ on a pye-unicam SP3-300 spect. using (KBr discs). ¹HNMR spectra were applied to detect protons (¹H) (using Bruker at 600 MHz, with TMS). Determination of melting points of newly product were done using Philip Harris melting point apparatus.

Synthesis of 4-[(E)-(3,4-dihydroxybenzylidene)amino]-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide

Preparation of Schiff base was obtained by mixing Sulfamethoxazole drug (2.0 mmol, 0.50 g) diluted in (15 ml) of methanol and 3,4-dihydroxy benzaldehyde (about 0.27 g, 2 mmol) in equal amount of ethanol, 3 drops of glacial acetic acid gathered to the mixture and stirring refluxed for (3 h.) , then left to cool and dried. The product was re-crystallized from

ethanol and orange product Yield 4-[(E)-(3,4-dihydroxybenzylidene)amino]-N-(5-methyl-1,2-oxazol3yl) benzenesulfonamide.

Color: Orange

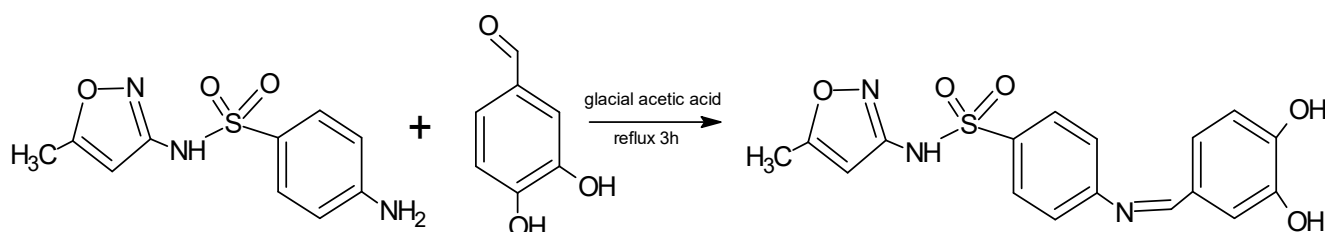
Yield: 78%.

M.p.: 177-179 Silesian.

FT-IR (KBr, ν , cm^{-1}): 3472-3325 (N-H); 3276-3240 (O-H); 3071 with 3016 (CH,Ar-H); 2960 (C-H, aliphatic), 1660-1592 (C=C and C=N).

^1H NMR (600 MHz, DMSO- d_6 , δ , ppm): 2.28 (s, 3H, CH₃); 6.06(s, 1H, Hoxazole); 7.81-6.57 (m, 7H, Ar-H); 8.37 (s, 1H, CH=N); 9.77 (s, 1H, NH); 10.25(s, 2H, OH)

Anal.. to C₁₇H₁₅N₃O₅S: C, 54.68; H, 4.04; N, 11.25; S, 8.58. Found: C, 54.37; H, 4.35; N, 11.42; S, 8.40 %.



Scheme 1: Synthesis of 4-[(3,4-dihydroxybenzylidene)amino]-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide

Antimicrobial activity

This activity was assigned against three bacterial types (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*) which obtain from central researches laboratory in Veterinary medicine college, University of Basrah, by using the paper disc-agar diffusion technique (8). Overnight cultures of selected bacteria were kept at 37°C for 24 h. in the incubator. Inhibition zone values of the compound tested for all the bacteria. On the other hand three serial dilutions (dimethylsulfoxide DMSO) with concentrations (50,100,200 $\mu\text{g/ml}$) of synthesized compound. Vancomycin was used for comparison. Filter paper discs 6 mm (Whatman, no. 3) were impregnated with 20 ml of each of the different dilutions. The discs were allowed to remain at room temperature until complete diluents. Discs with natural and synthesized products placed onto the surface of Muller Hinton agar. After 24h of incubation, The antibacterial activity was evaluated according to its zone of inhibition (mm).

RESULTS AND DISCUSSION

Chemistry

Structural modification of sulfamethoxazole drug by reaction of 4-amino-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide and 3,4-dihydroxy benzaldehyde in 1:1 ratio to yield new Schiff base derivative. The IR spectrum confirms the existence of the azomethine group (CH = N) extending about 1592 cm⁻¹ with a sharp area.

¹H NMR spectrum of synthesized compound show chemical shift at δ 2.28 ppm due to methyl group. The spectrum show signal of oxazole ring proton at δ 6.06 ppm, the region at δ 7.81-6.57 ppm due to aromatic protons. The ¹H NMR spectrum show signal to azomethine group (CH=N) at δ 8.47 ppm (9). The spectrum synthesized compound show signal in δ 9.77 ppm due to NH proton. ¹H NMR spectra of synthesized compound display signal in δ 10.25 ppm by phenolic OH (10), Figure 3.

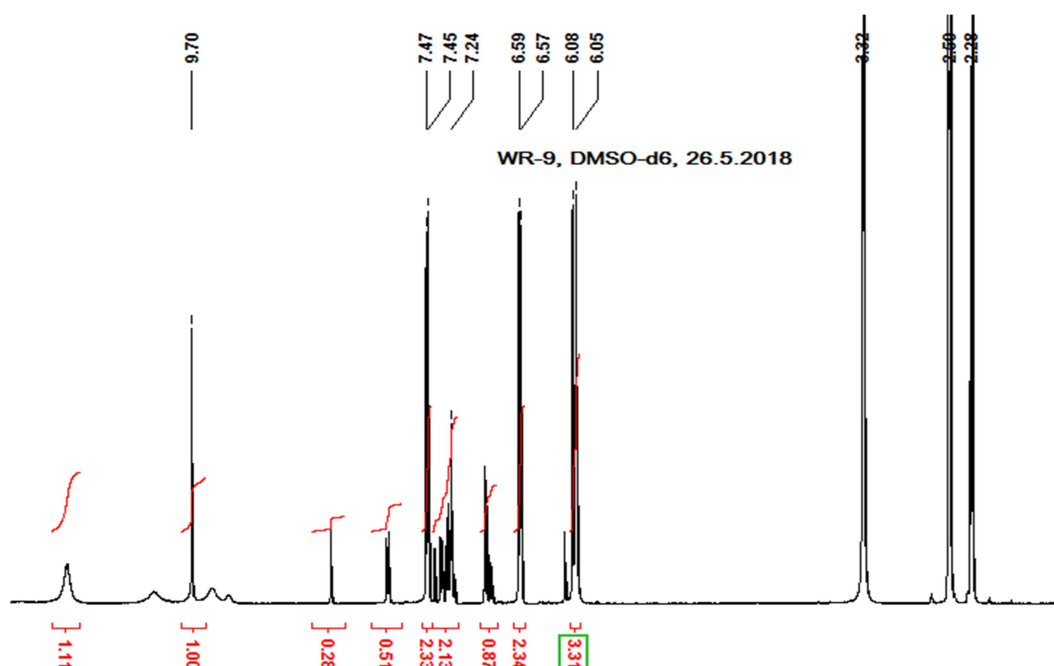


Figure 2: ¹H NMR spectrum of 4-[(3,4-dihydroxybenzylidene)amino]-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide

Antibacterial activity

Preliminary study of antimicrobial activity were done on three various strains of bacteria one Gram positive and two Gram negative displayed that the final compounds significant activity compared with standard drug vancomycin. The results of the antibacterial assays are given in Figure 3 and Table 1.

Table 1: Antibacterial Activity of new sulfamethaxazole derivative

Bacteria	Inhibition zone of antibacterial sensitivity test to compound (mm)			
	Standard Van.Conc.(µg/ml)(disk)	New sulphamethaxazole derivative conc.(µg/ml)		
	30	50	100	200
<i>E. coli</i>	13	-	8	10
<i>S. aureus</i>	25	9	14	18
<i>K. pneumonia</i>	-	14	17	22

The synthesized compound give good or moderate antibacterial activity against all the tested strains compared to the standard antibiotic vancomycin.

Although sulfamethoxazole is important as a useful drug, But it is becoming less effective as the bacteria's resistance to its action expands. So nowadays Sulfamethoxazole is combined with some substance such as trimethoprim, a combination product known as Bactrim or Septra (11). Therapy with this combination in case of acute otitis media (AOM) in different ages occurred due to susceptible *S. pneumoniae* or *H. influenza* (12,13).

It is evidenced by the results the sulfamethaxazole modified is effective against both gram negative and positive bacteria such as *E coli*, *Klebsiella* and *Staphylococcus*, Figure 3 and Table 1.

Although sulfamethaxazole is ineffective against *Staphylococcus* bacteria, the new modified compound has high efficacy, and because of this, the new compound may be an alternative to co-trimonoxazole for high efficacy against *staphylococcus* bacteria.

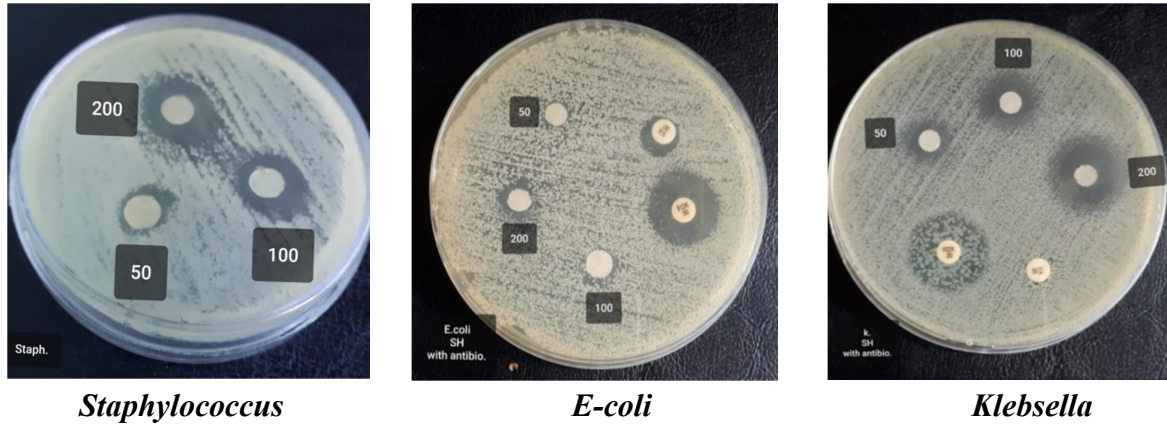


Figure 3: Antibacterial activity of sulfamethaxazole derivative against

تحضير والفعالية المضادة للبكتريا لمشتق عقار السلفاميثا كسازول

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الخلاصة

أدى تكثيف عقار السلفاميثا كسازول مع 3,4-dihydroxy benzaldehyde إلى إنتاج مشتق جديد من قاعدة شيف بحصيلة انتاجية جيدة. تم تشخيص المركب المحضر بالطرق الطيفية المتمثلة بطيف الاشعة تحت الحمراء IR و طيف الرنين النووي المغناطيسي للبروتون NMR للمركب الجديد باستخدام الرنين المغناطيسي النووي 600 ميغاهرتز. تم فحص المركب المحضر بحثاً عن النشاط المضاد للبكتيريا ضد عدد من انواع البكتريا الموجبة والسالبة الجرام. أظهر المركب الجديد نشاطاً قوياً مضاداً لبكتريا *Klebsiella pneumonia* مقارنة بعقار Vancomycin والذي لم يظهر اي نشاط ضد هذا النوع من البكتريا، كما أظهر المركب المحضر نشاطاً "متوسطاً" ضد *Escherichia coli* و *Staphylococcus aureus*.

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