Synthesis and microbial activity of a new azo-Schiff base

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A B S T R A C T

As a result of the development of microbes and the emergence of antibiotic-resistant types over time. There is a need to prepare new antibiotics that help the immunity of living organisms against microbes. The new studies showed that azo compounds and Schiff bases have biological activity as anti-bacterial and anti-fungal agents. Therefore this study included, the synthesis of 2-hydroxy-5(4-nitrophenyl)diazinyl) benzaldehyde compound by diazonium coupling of para-nitro-aniline with salicylaldehyde. Where the Schiff base was prepared between the prepared azo compound with 4-chlorobenzylamine in the alcoholic medium and then the activity of the prepared compound against the microbial activity, bacterial activity and cytotoxicity of blood, was studied. The study showed that the prepared compound has biological activity against Candida albicans and bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus lentus)*. Sulfadiazine was used as a reference. This study showed that the prepared azo-Schiff compound can act as an antibiotic.

1. Introduction

Schiff bases are condensation products of primary amines with carbonyl compounds. Schiff bases are compounds bearing an imine or azomethine functional group (–C=N–) that have important biological effects such as anti-inflammatory.[1] analgesic, and antimicrobial [2] The literature showed that compounds containing the Schiff group have a biological activity to inhibit the fungal[3]. Schiff bases play an important role in the preparation of many organic chemical compounds that are used in manufacturing and biological processes such as formazans, 4-thiazolidinines and benzoxazines [4],[5]. They are also used in many environmentally friendly chemical manufacturing processes, especially in the preparation of heterocyclic organic compounds.

The pharmacological use of Schiff compounds arises from the discovery of the antibacterial effect of Prontosil on streptococcal [6].

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The azo compound has a chemical structure that has (R-N=N-R) in its skeleton [7]. Azo compounds play an important role in several applications. They are used as dyes, inhibitors of protein and RNA formation, anti-bacterial pesticides, antiviral anti-inflammatory and anti-cancers [8],[9]. Also, Azo compounds are used in the manufacture of many pharmaceutical preparations [10].

2. Expermintal

2.1. Material and Methods:

The chemicals shown in scheme 1 and pure Sulphadiazine were from (Alpha, BDH, Riedel-de Haen and VWR). Stuart melting point apparatus (SMP3) was used to determine the melting point at the University of Basrah college of pharmacy. IR Affinity-1 Spectrophotometer was used to record FT-IR spectra using Potassium Bromide disc at the University of Basrah. Anti-bacterial and cytotoxicity of blood were recorded in the Microscopic Research Laboratory at the College of Pharmacy, University of Basrah. 1H NMR spectra of the synthesized compound were recorded on a Bruker-Avance (400 MHz) and CDCl3 solvent and TMS as an internal standard in the Department of Chemistry, College of Education, University of Basra .

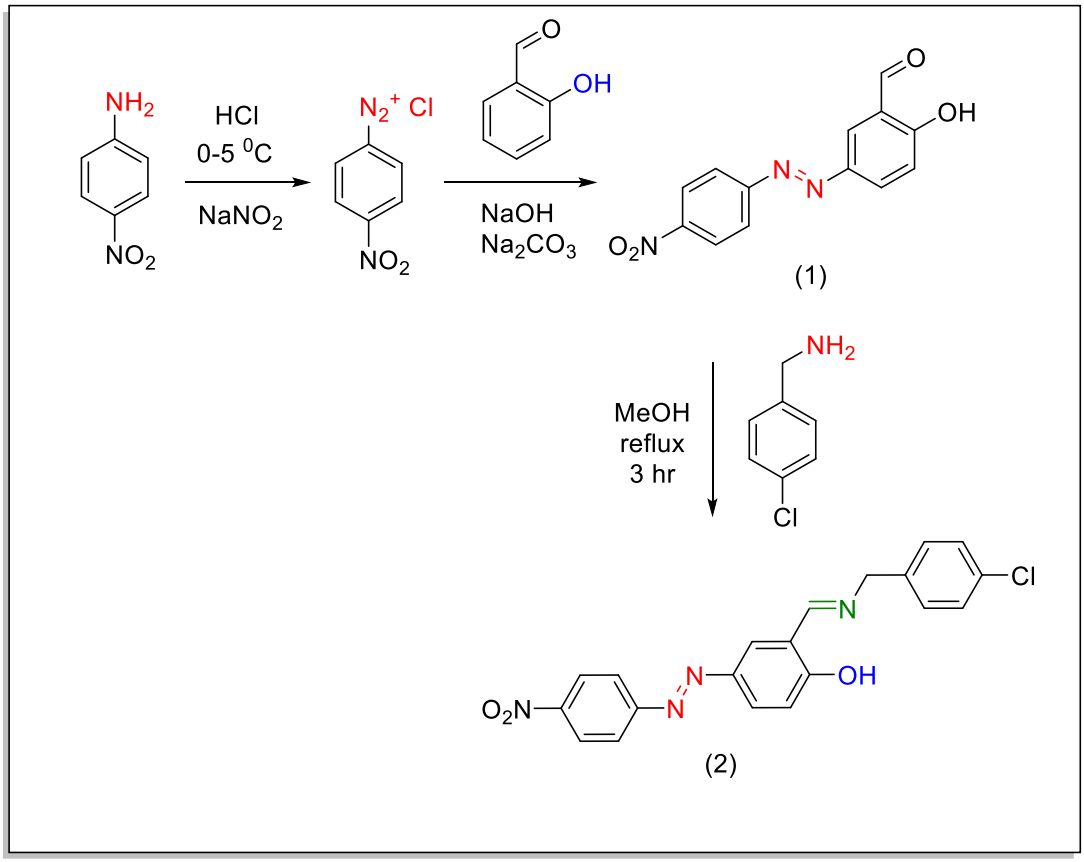
2.2. Synthesis procedure:

Azo salicylaldehyde **1** was prepared according to the procedure as follows [11]; a mixture of p-nitro aniline (1 mmol) in 2ml of hydrochloric acid, and 10 ml of water was heated to 70 °C to be dissolved completely. The clear solution was poured into an ice-water mixture and was diazotized with 1 mmol of sodium nitrite dissolved in 1 ml of water at 0-5 °C for 30 mins. The cold diazo solution was added to the solution of salicylaldehyde (1 mmol) in water (20 ml) containing sodium hydroxide (0.04g) and sodium carbonate (0.37 g). during the period of 1.5 h at 0-5 °C. During the adding process, diazo solution was vigorously stirred. The product was collected by filtration and washed with water and recrystallized from ethanol.

Schiff base **2** was prepared by condensation of azo salicylaldehyde **1** (0.001 mol) with 4-chloro benzyl amine (0.001 mol) in methanol (20 mL) and the mixture was heated to reflux for 3 hr. The progress of reaction was monitored by TLC. The product was separated as red-coloured which was filtered, dried, and recrystallized from ethyl acetate [12]

2-hydroxy-5-((4-nitrophenyl)diazenyl)benzaldehyde (1) : ($C_{13}H_9N_3O_4$), Molecular Weight: 271.23 m.p= 146-147 °C, orange-dark, IR (KBr): 1600 (C=C), 1523 (N=N); 1643 (aldehydic C=O);3039(Ar-H Aromatic), 3414 cm^{-1} (Ar-OH). ^{1}H NMR (400 MHz, DMSO) δ 11.80 (s, 1H), 10.38 (s, 1H), 8.50 – 8.35 (m, 2H), 8.24 (d, J = 2.6 Hz, 1H), 8.15 (dd, J = 8.9, 2.6 Hz, 1H), 8.10 – 7.99 (m, 2H), 7.23 (d, J = 8.9 Hz, 1H).

2-(3-(4-chlorophenyl)prop-1-en-1-yl)-4-((4-nitrophenyl)diazenyl)phenol (2) : ($C_{20}H_{15}ClN_4O_3$), Molecular Weight: 394.82, m.p = 156-158 °C (red) IR (KBr): 1585 (C=C), 1489 (N=N), 1635 (C=N), 3028 (Ar-H
Aromatic), 3400 (Ar-OH), ^{1}H NMR (400 MHz, DMSO) δ 14.24 (s, 1H), 8.89 (s, 1H), 8.51 – 8.30 (m, 2H), 8.17 (d, *J* = 2.6 Hz, 1H), 7.99 (dd, *J* = 9.2, 1.7 Hz, 3H), 7.46 (q, *J* = 8.7 Hz, 4H), 6.88 (d, *J* = 9.2 Hz, 1H), 4.88 (s, 2H), ^{13}C NMR (400 MHz, DMSO) δ 57.3 (CH_2-N), 166.7 (C-OH), 155.6 (HC=N), 147.5 (HC-N=N), 142.6 (N=N-CH), 116.5-136.5 (C=C Aromatic) ppm.



Scheme 1 : preparation azo salicylaldehyde and Schiff base .

2.3 Antimicrobial activity (agar diffusion method) :

Antimicrobial activity of the two synthesized compounds were determined by the agar diffusion method[13], *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus lentus*, *Candida albicans*, are the microbial Stock solutions of compounds were diluted in distilled and sterilized water Then the sample was distributed on an agar dish . For antibacterial activity, nutrients agar was used[14]. The antibiotic sulfadiazine is used as a reference antibacterial agent. Inoculated plates were then incubated at 37 °C for an antibacterial activity for 16 hr. after that we measured the inhibition zone.

2.4. Cytotoxicity of blood:

To measure cytotoxicity of blood of the azo-Schiff compound **2**, three concentrations of the compound were prepared (1000 ppm, 500 ppm and 250 ppm)[15] . The blood solution was prepared by taking 2 ml of human blood and adding it to 20 ml of the normal saline (N.S) solution. In the test tube, 2ml of the blood solution was added to 0.2 ml of sample with different concentrations. Then two ml of blood solution was added to 0.2ml of the appropriate solvent in different concentration DMSO. The samples are placed in a water bath at 37 °C (1-3 hours). The solution was shaked every ten minutes.

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3. Results and Discussion

3.1 Synthesis of Azo-Schiff base

The azo compound **1** was prepared from salicylaldehyde and 4-nitroaniline in an acidic medium, then the solution was neutralized to obtain the azo dye **1** as an orange-dark solid product. Salicylaldehyde was used since it has hydroxyl group which play an important role in the biological activity. After that, the azo compound **1** was treated with an alcoholic solution of 4-chlorobenzylamine, and heated at reflux for three hours to give azo-Schiff base as a red precipitate, as shown in **Scheme 1**. The obtained date result ,IR,^{1}H-NMR and ^{13}C-NMR , proved the prepare compounds .

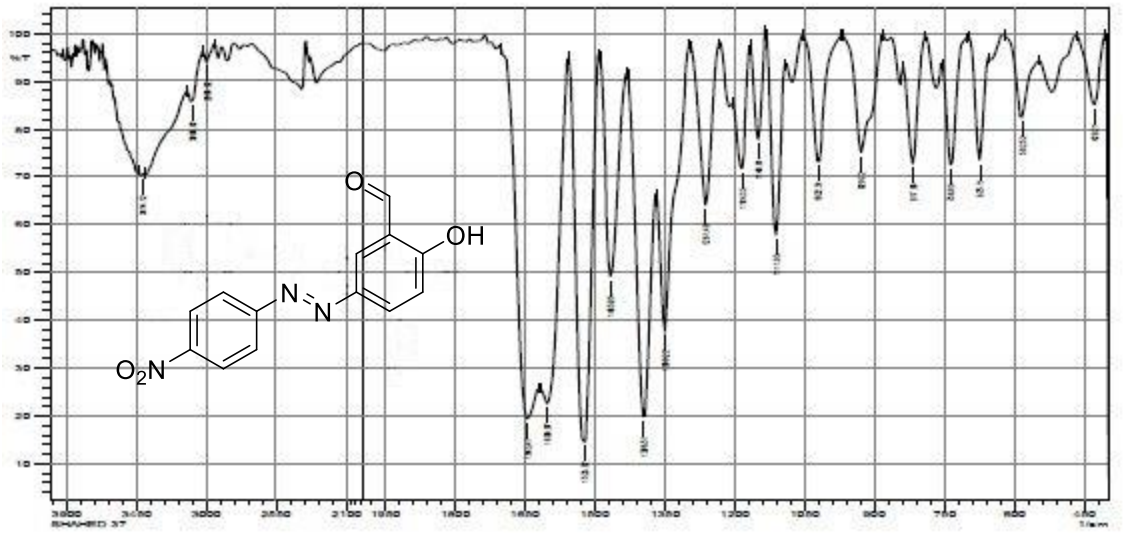


Fig.1 IR-spectrum of Azo salicylaldehyde 1

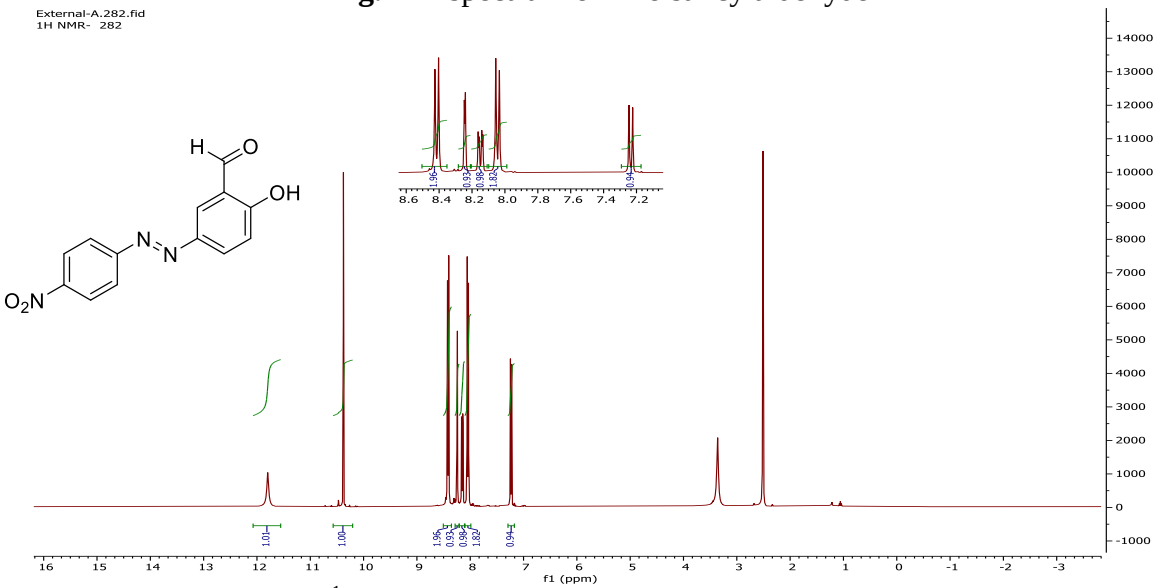
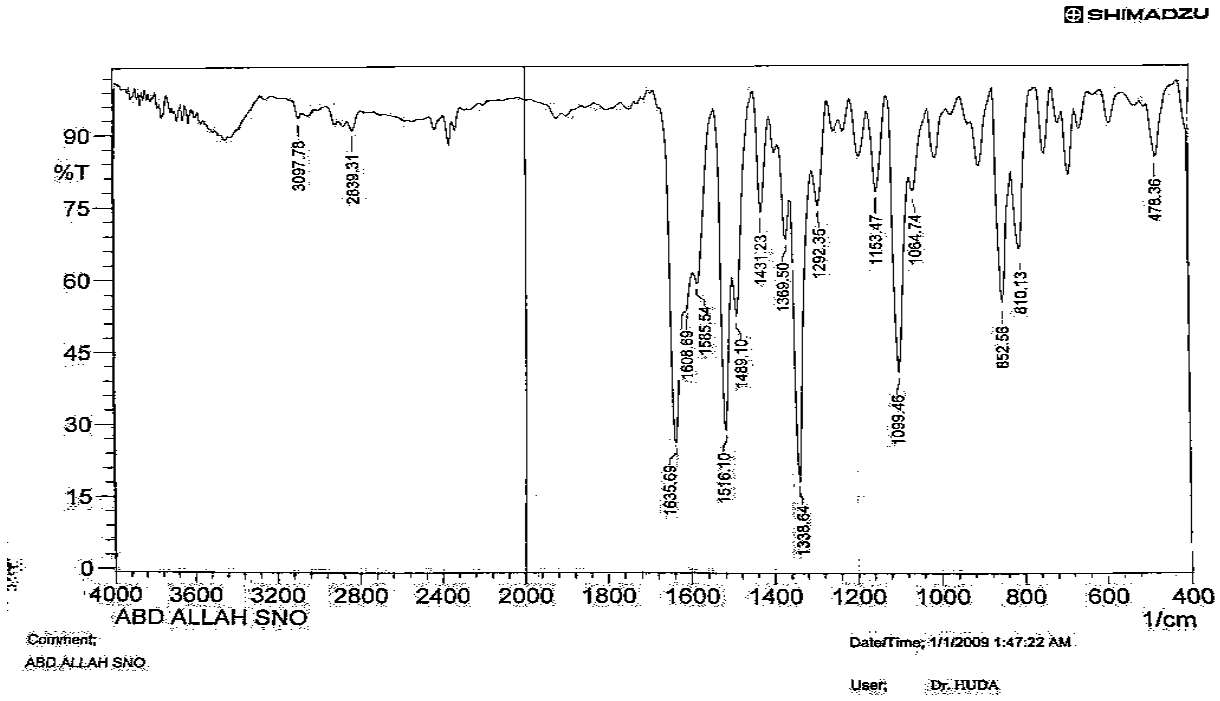


Fig.2 ^{1}H NMR -spectrum of Azo salicylaldehyde 1

Fig.3 IR-spectrum of Azo-Schiff (2)



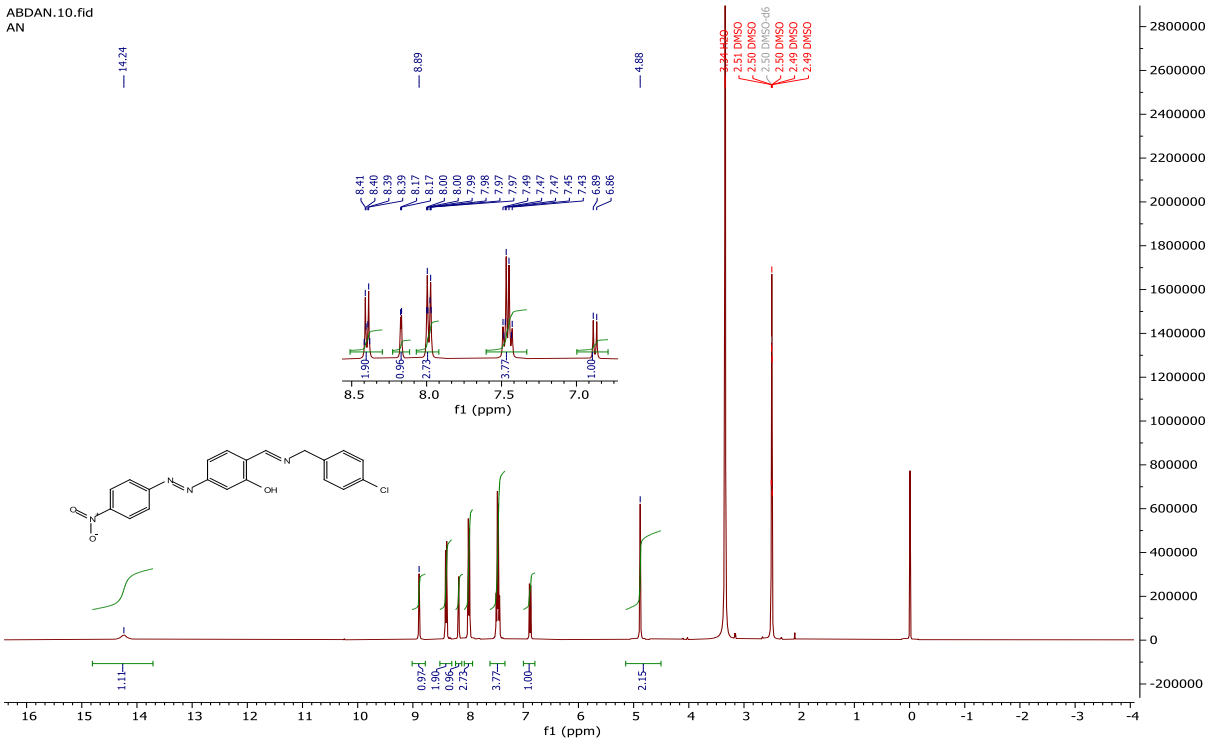


Fig.4 ^{1}H-NMR-spectrum of Azo-Schiff (2)

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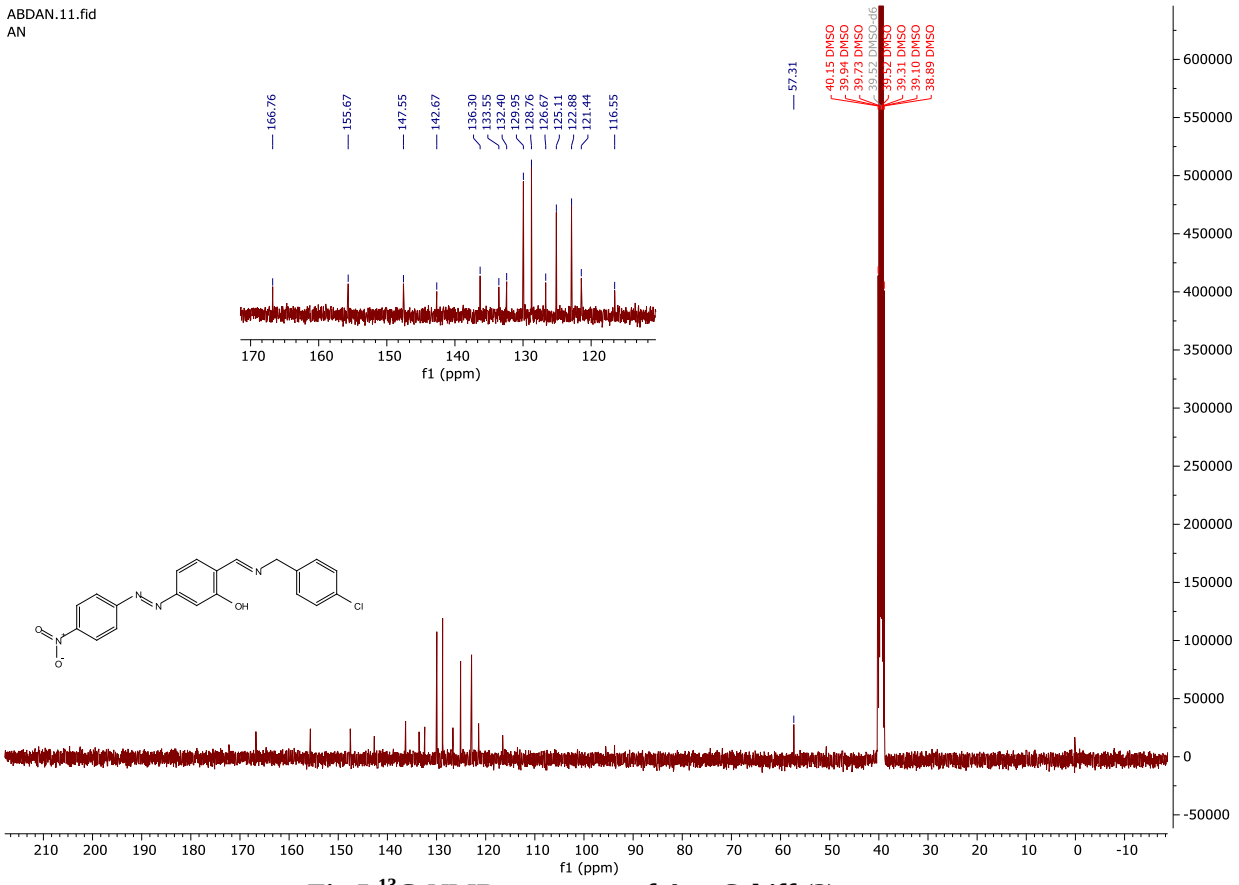

Fig.5 ^{13}C-NMR-spectrum of Azo-Schiff (2)

3.2 Biological activity

The inhibition zone results of the prepared azo-Schiff compound **2** are depicted in **Table 1**. The results revealed that the tested compound showed moderate to good bacterial and fungal inhibition.

Table 1 : The biological activity of compound **2** and the reference

Comp.	**Inhibition zone (mm)**			
	Staphylococcus lentus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Azo-Schiff (2)	9	14	14	12
Sulphadiazine	14	11	15	13

Gram +ve Bacteria: *Staphylococcus lentus*, *Pseudomonas aeruginosa*.
Gram -ve Bacteria: *Escherichia coli*.
Fungal : Candida albicans .

The results in Table 1 showed that the azo-Schiff compound gave higher inhibition activity (14 mm) compared to the standard reference sulfadiazine (11 mm) against Pseudomonas aeruginosa and similar activity against Escherichia coli and Candida albicans (14 and 12 respectively) compared to the sulfadiazine (15 and 13 respectively). However, the prepared azo-Schiff

compound 2 gave less inhibition activity against *Staphylococcus lentus* (9 mm) compared with sulphadiazine (14 mm).
The biological activity of new azo-Schiff compound was compared with the closest similar compound 3-(Benzyl)-2-(4-oxo-2-phenylquinazolin-3(4H)-ylamino)quinazolin-4(3H)-one (reference), which was higher than the reference. Previous study showed that the reference compound gives antimicrobial activity against (*E.coli*) is 10 mm and against (*Pseudomonas aeruginosa*) is 7 mm[16] .

3.3 Cytotoxicity of blood:

The cytotoxicity of azo-Schiff **2** was measured at three concentrations (1000 ppm, 500 ppm and 250 ppm). It was observed that azo-Schiff 2 base gave positive hemolysis results on blood solution at 500ppm and 250ppm.

4. Conclusion

In this study, a new azo-Schiff compound was prepared through the reaction of azo aldehyde with 4-chloro benzyl amine. The biological activity of the prepared compound was studied against three types of bacteria (*Staphylococcus lentus, Pseudomonas aeruginosa and Escherichia coli*) and one type of fungi (*Candida albicans*) where sulfadiazine was used as reference. the results showed that the prepared compound had higher inhibition activity than of sulfadiazine against *Pseudomonas aeruginosa*, and similar activity for the others. Also, this study has showed the prepared compound had no toxicity at concentrations (500 ppm and 250 ppm).

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نموذج الملخص باللغة العربية لمجلة ابحاث البصرة (العلميات)

تحضير ودراسة الفعالية البايلوجية لمركب جديد من الازو شف

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معلومات البحث

الاستلام يوم شهر سنة
القبول يوم شهر سنة
النشر يوم شهر سنة

الكلمات المفتاحية

2-هيدروكسي -5 (4-نيتروفينيل) ديازينيل) بنزالديهايد ، 4-كلورو بنزيل أمين ، آزو قاعدة شف ، الفعالية البايلوجية .

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الملخص

نتيجة لتطور الميكروبات وظهور أنواع مقاومة للمضادات الحيوية مع مرور الوقت. لذا هناك حاجة لتحضير مضادات حيوية جديدة تساعد على مناعة الكائنات الحية ضد الميكروبات. أظهرت الدراسات الجديدة أن مركبات الآزو وقواعد شيف لها نشاط بيولوجي كعوامل مضادة للبكتيريا ومضادة للفطريات. لذلك تضمنت هذه الدراسة تخليق 2-هيدروكسي -5 (4-نيتروفينيل) ديازينيل) بنزالديهايد عن طريق اقتران ديازونيوم من بارا-نيترو-أنيلين مع السلسلدهايد . حيث تم تحضير قاعدة شيف بين مركب الآزو المحضر مع 4-كلورو بنزيل أمين في الوسط الكحولي ثم تمت دراسة نشاط المركب المحضر ضد النشاط الجرثومي والنشاط البكتيري والسمية الخلوية. أظهرت الدراسة أن المركب المحضر له نشاط بيولوجي ضد الفطر من نوع *Candida albicans* والبكتيريا من نوع (*Escherichia coli*، *Pseudomonas aeruginosa*، *Staphylococcus letus*). تم استخدام سلفاديازين كمرجع. أظهرت هذه الدراسة أن مركب azo-Schiff المحضر يمكن أن يعمل كمضاد حيوي ..

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