

Synergistic and Antagonistic Effects of Azo-Schiff Base with Some Antibiotics on Gram-Positive Bacterial Isolates

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Abstract

Antibacterial resistance has brought an urgent need to find a solution to the problem of antibacterial resistance and increase the effectiveness of antibiotics. Therefore, our current study was aimed to test the three concentrations viz., 0.5 mg/ml, 5 mg/ml and 50 mg/ml of Azo-Schiff base against Gram-positive bacteria—Bacillus cereus, Micrococcus lutes, Staphylococcus aureus—to compare the effects of Schiff base alone or along with antibiotics on the viability of bacteria. Our results showed no antibacterial activity of Schiff base (L₂) against three bacterial strains except S. aureus at concentration of 50 mg/ml. Synergistic effects of Schiff base was observed against B. cereus with only antibiotics CN₁₀, but antagonistic effects was observed at all concentrations with all antibiotics.

Keywords: Resistance, Schiff base, synergistic effect, antagonistic effect, antibacterial

INTRODUCTION

The increase in mortality rate associated with infectious disease is related to bacterial resistance to the existing antibiotics. Thus the demand for new effective antibacterial agents is a continuous phenomenon. Schiff bases constitutes one of the cases of chemical compounds which hold great promise [1, 2]. These compounds are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities [3]. They show promising applications in medicine as antibacterial, antifungal, antiviral, anticancer and antimalarial agent [4, 5]. Also these compounds has been used for industrial purposes such as pigments, catalysts etc. [6, 7].

Azo-Schiff bases having imine groups (C=N) are formed by condensation reaction of primary amines (NH₂) with aldehydes (CHO) or ketones (CO) [8]. Aromatic Azo compounds (R=R) are usually stable; that fact can be explained by the side group around the azo bond that helps to stabilize the (N=N) group by making it a part of a delocalized system often absorbing visible frequencies of light [9].

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Received Date: March 17, 2021

Accepted Date: May 20, 2021

Published Date: August 27, 2021

Citation: Salimah Aboulqasim Ali, Najat Ramadan Elzawi, Abdelhamid Mohamed Elyassiry. Synergistic and Antagonistic Effects of Azo-Schiff Base with Some Antibiotics on Gram-Positive Bacterial Isolates. Research & Reviews: A Journal of Microbiology & Virology. 2021; 11(2): 25–31p.

In the present work, we have been interested in the design synthesis of Azo-Schiff bases [2 – [(z)-(2- mercapto-5-[(z)- (2-methyl) phenyl] diazenyl] imino)methyl] phenol. We have also tested them alone on the activity of *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*, and tested along with some antibiotics on the activity of bacteria.

MATERIALS AND METHODS

All chemicals were purchased from (OXOID) chemical products company, and they were employed for the preparation of Azo-Schiff base. Antibiotics were of analytic grade and used without further purification.

Synthesis of Azo-Schiff Base (C₁₉H₁₅N₃O₃S); L₂

Azo-Schiff base was prepared according to the literature procedure by El-ajaily et al. [10]. 2-aminobenzoic acid (1.37 g, 0.01 mol) was dissolved in 20 cm³ nitrite (0.76 g). At the same temperature Schiff base (2.29 g, 0.01 mol) was added while stirring followed by addition of sodium hydroxide solution (4 g, 0.1 mol). The mixture was made up to 100 cm³ by adding absolute ethanol and kept in ice bath at 0°C. The obtained reaction mixture was stirred for 40 min and then isolated liver-color solution was left overnight for 24 h in the refrigerator. The obtained Azo-Schiff base was acidified by dilute hydrochloride acid until pH range of 2–4 was reached. Then the obtained Azo-Schiff base was filtrated and washed by distilled water, then it was dried and recrystallized from suitable solvent.

Different concentrations of Schiff base (0.5 mg/ml, 5 mg/ml, 50 mg/ml) were prepared by dissolving Schiff base in 10% methyl sulfoxide (DMSO).

Biological Activity

Three isolates of Gram-positive bacteria were used in the present study—*Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*—obtained from the microbial museum, Department of Zoology, Sabha University, Libya. They were prepared from overnight grown cultures (24 h) in agar media. Turbidity was adjusted equivalent to 0.5 McFarland unit. Then 100 µl of inoculums were spread on the surface of Mueller Hinton Agar (OXOID) plates with a sterile glass spreader.

Antibacterial Test

Azo-Schiff Base Test

The Azo-Schiff base L₂ was tested in vitro for antibacterial activity against three species of bacteria by well diffusion method [11]. Saturated discs (6 mm diameter) with different concentrations (0.5 mg/ml, 5 mg/ml, 50 mg/ml) of Azo-Schiff base were placed on the surface of petri dishes containing bacteria grown on Mueller Hinton agar [4]. The petri dishes were placed at 37°C for 24 h. At the end of period, the inhibition zones formed on the media were measured (in millimeters) with a zone reader. DMSO was used as control. DMSO had no effects on the bacteria [12].

Azo-Schiff Base and Antibiotics Test

Synergistic and antimicrobial effect of Schiff base with antibiotics was tested according to Majeed and Ghanim [13]. About 10 µl of Schiff base was added to saturated discs of the antibiotics with a known concentration.

Then the discs were placed on the surface of the agar after being inoculated with bacteria. The petri dishes were incubated for 24 h at temperature 37°C. The formed inhibition zones were measured in millimeters. The values in our results were given as a mean of three replication.

RESULTS AND DISCUSSION

In the present study, Azo-Schiff base were synthesized according to previous study by El-ajaily et al. [5]. The preparative illustration is given in Figure 1.

In the present work, three concentrations viz., 50 mg/ml, 5 mg/ml, 0.5 mg/ml of the Azo-Schiff base L₂ were tested on three isolates of *B. cereus*, *M. luteus* and *S. aureus*. The results are shown in Table 1 and Figure 2.

The antibacterial activities of Azo-Schiff base showed no activity against *B. cereus* and *M. luteus*; while weak activity was observed against *S. aureus* only at a concentration of 50 mg/ml where the diameter of inhibition zone was recorded as 8 mm.

Table 1. Effects of Azo-schiff base on three bacterial isolates.

Conc. of Schiff base (mg/ml)	Bacterial strains		
	Zone of inhibition		
	<i>B. cereus</i>	<i>M. luteus</i>	<i>S. aureus</i>
50	R	R	8±1 ^{b,c}
5	R	R	R ^a
0.5	R	R	R ^a
Control (DMSO)	NF	NF	NF

Penicillin (P_{10}), Streptomycin (S_{10}), Vancomycin (VA_5), Ampicillin (AMP_{10}), Gentamycin (CN_{10}), Cefamandole (MA_{30}), Clindamycin (DA_2), Chloramphenicol (C_{10}) R=Resistance, NE=No effect.

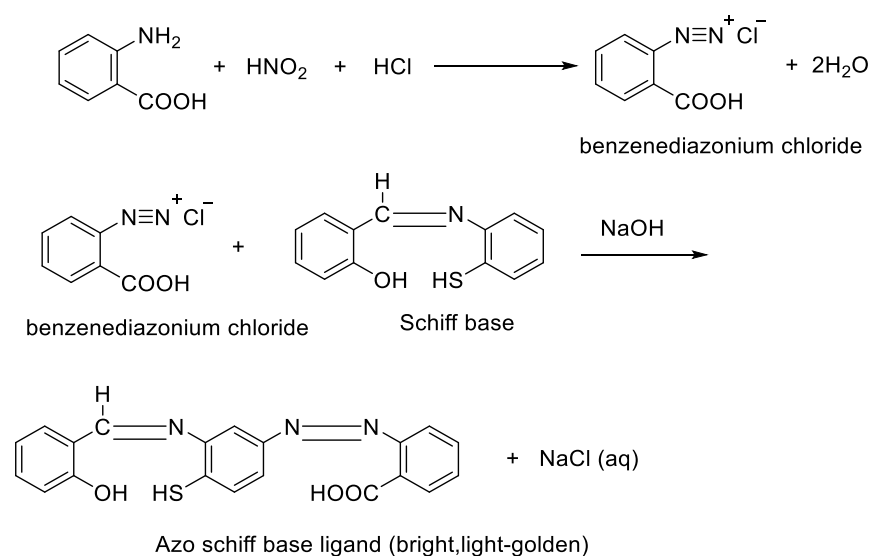


Figure 1. Synthesis of Azo-Schiff base ligand (Bright-Golden).

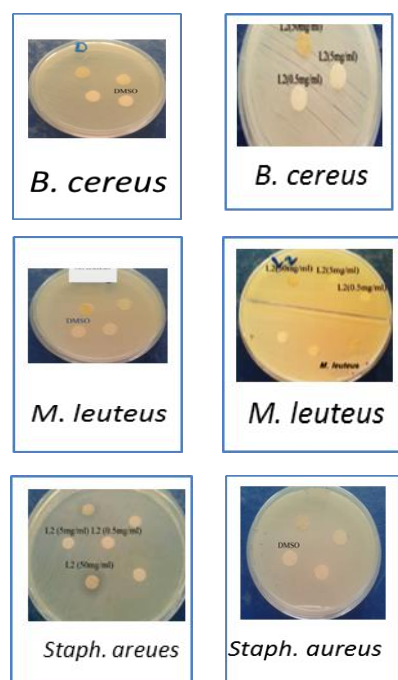


Figure 2. Control (DMSO) (A) and effect of schiff base on bacterial strains (B).

The second part of this study included the investigation of the synergistic and antagonistic effects of this base with eight antibiotics against Gram-positive bacteria. The obtained results were summarized in Table 2 and Figure 3. The antibiotics susceptibility test showed that *B. cereus* and *M. luteus* were sensitive to all the tested antibiotics. But *S. aureus* was moderately sensitive only to DA₂, CN₁₀ and C₁₀, while it was very weakly sensitive to VA₅ and S₁₀. A synergistic effect of Azo-Schiff base with antibiotics was observed only with antibiotic CN₁₀, while antagonistic effects of base was reported with all antibiotics (Figure 4). *B. cereus* and *M. luteus* showed changes in sensitivity to antibiotics as a result of mixing the base with all antibiotics. We observed that it has a pattern of antibiotic resistance with P₁₀, AMP₁₀, MA₃₀ and C₁₀. Mixture of the Schiff base with AM₁₀ increases the effectiveness of this antagonist, but this resistance has not changed with P₁₀, AMP₁₀ and MA₃₀. These results were in agreement with previous studies that suggested *S. aureus* as highly resistant to antibiotics [14]. Some bacterial strains are naturally resistant to some antibiotics due their physiological characteristics and have multiple routes to resistance to antibiotics. Resistance can result from modification of an antibiotics target or from functional bypassing of that target, also it can be impermeability, efflux or enzymatic activation [14]. Antibiotic resistance can either be inherent, which occurs when a bacterium that was originally sensitive to an antibiotic develops resistance, in which resistance genes can be transformed on plasmid or chromosome or occur as a random spontaneous chromosomal mutation [14, 15]. In our study, we did not observe a strong activity of Schiff base on resistance of bacteria to the antibiotic except with CN₁₀; it was synergistic effect of Azo-Schiff base with this antibiotic on the bacteria. The antagonistic effects were moderate showing approximately at all concentrations with antibiotics.

Table 2. Synergistic and antagonistic effects of concentrations of Schiff base (L₂) with antibiotics.

Bacteria	Control and Conc. of Schiff Base	Antibiotics							
		DA ₂	VA ₅	P ₁₀	AMP ₁₀	CN ₁₀	S ₁₀	MA ₃₀	C ₁₀
<i>B. cereus</i>	Control	11.1±1	11.66±1.52	14.33±1.15	14.33±0.57	18.33±2.08	13.66±2.08	14±2.64	11.66±1.15
	50 mg/ml	7.33±0.57*	11.66±1.15	10.66±1.52*	8.66±1.52*	19.66±0.57	13.33±1.52	11.66±1.52	11.66±1.15
	5 mg/ml	7.66±1.15*	11.66±0.57	7±0*	11.66±0.57*	19.66±0.57	13.33±2.08	11.66±2.08	11.1±1
	0.5 mg/ml	10.33±0.57	11.3±1.52	R*	12±1	19.66±0.57	12.33±2.51	R*	11±1
<i>M. luteus</i>	Control	11±1	10.66±0.57	16±1.73	16.66±1.52	22±2.64	13.66±1.52	14.66±2.30	15±1
	50 mg/ml	R*	10.66±0.57	17.33±1.52	14.66±0.57	18±1.73	8±1*	10.33±1.15	14.33±1.52
	5 mg/ml	R*	10.33±0.57	17.33±1.15	14.33±1.15	17.33±1.15*	8±1*	10.66±1.52	14.33±0.57
	0.5 mg/ml	R*	9.66±0.57	15.33±2.33	14.33±0.57	17.33±1.15	8±1*	10.66±0.57*	14.33±1.15
<i>S. aureus</i>	Control	15.33±0.7	11±1	R	R	18.33±1.15	11.66±1.52	R	13.33±0.57
	50 mg/ml	12.66±2.08*	10.33±0.57	R	R	17.33±0.57	10.66±0.57	R	10.66±1.15
	5 mg/ml	10.66±0.57	9.33±0.57	R	R	17.33±0.57	11±0	R	11±1.73
	0.5 mg/ml	10.3±0.57* g****	9.33±0.57*	R	7±0	18.66±1.52	11±0	R	11.33±1.52

R=Resistance, DA₂=clindamycin, VA₅=vancomycin, P₁₀=penicillin, AMP₁₀=ampicillin, CN₁₀=gentamycin, S₁₀=streptomycin, MA₁₀=cefamandole, C₁₀=chloramandole. *means the presence of significant differences at P≤0.05 of significance.

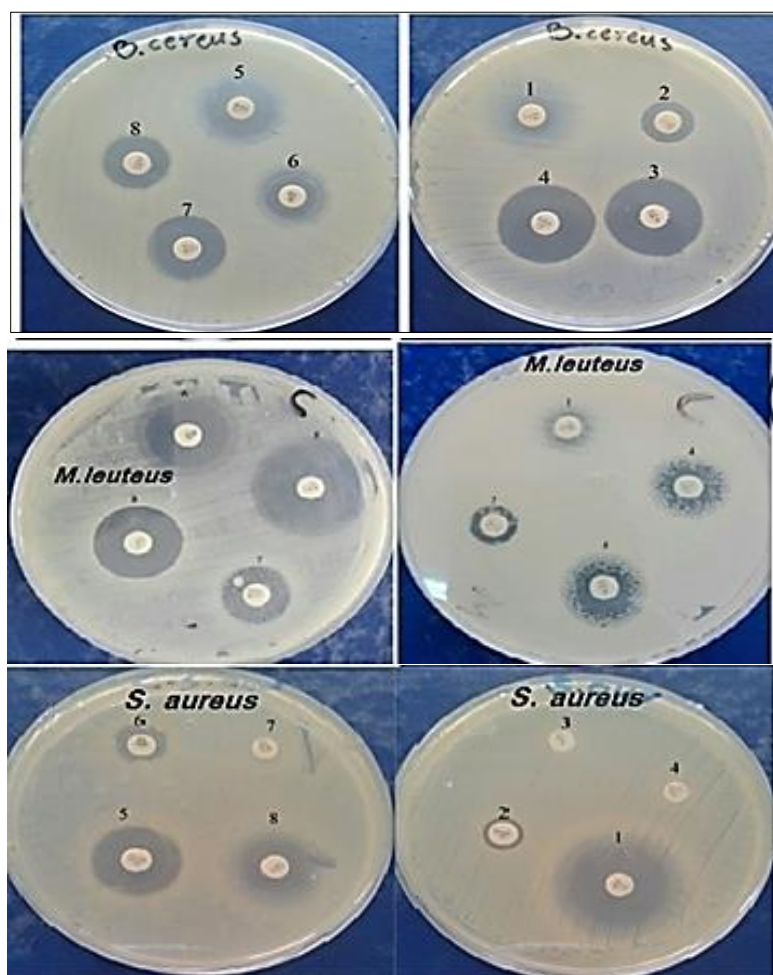
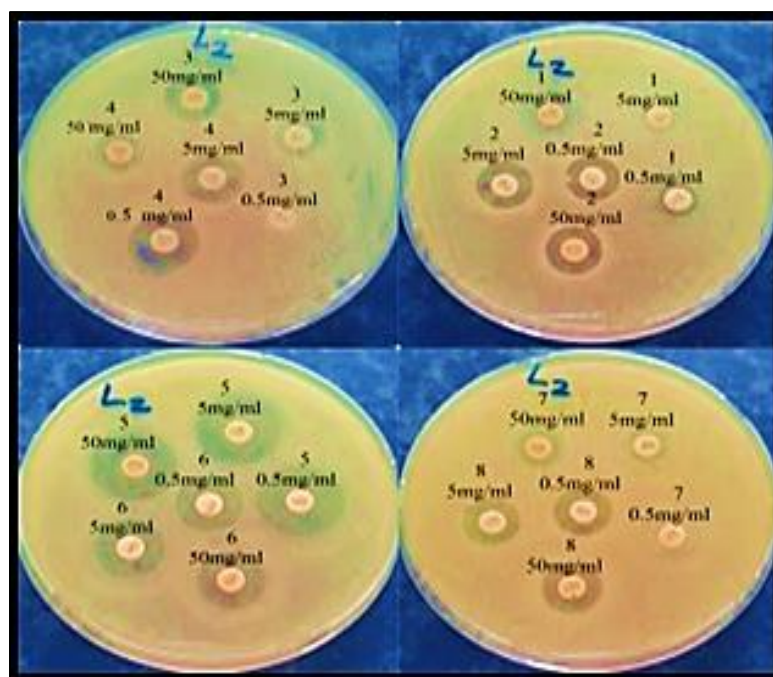


Figure 3. Effect activity of alone antibiotics (Control).



B. cereus

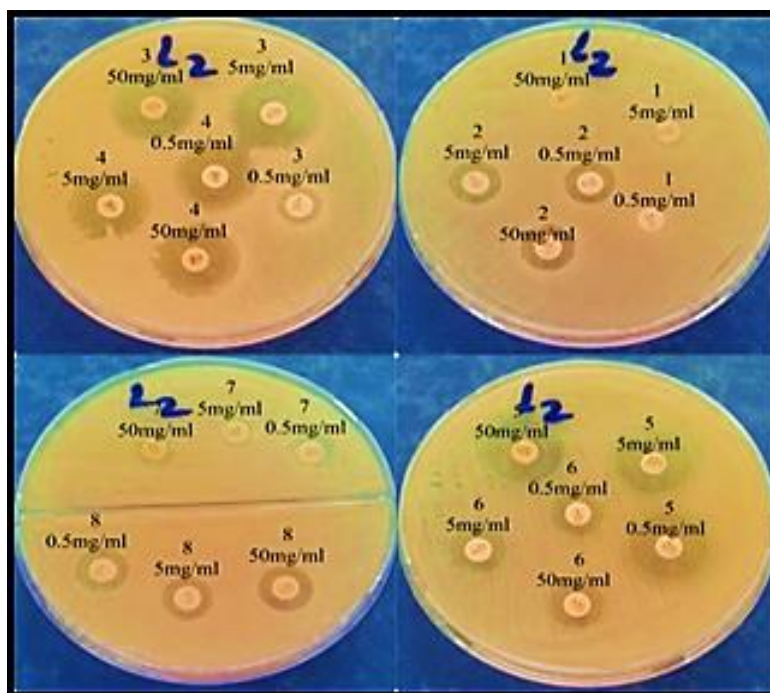
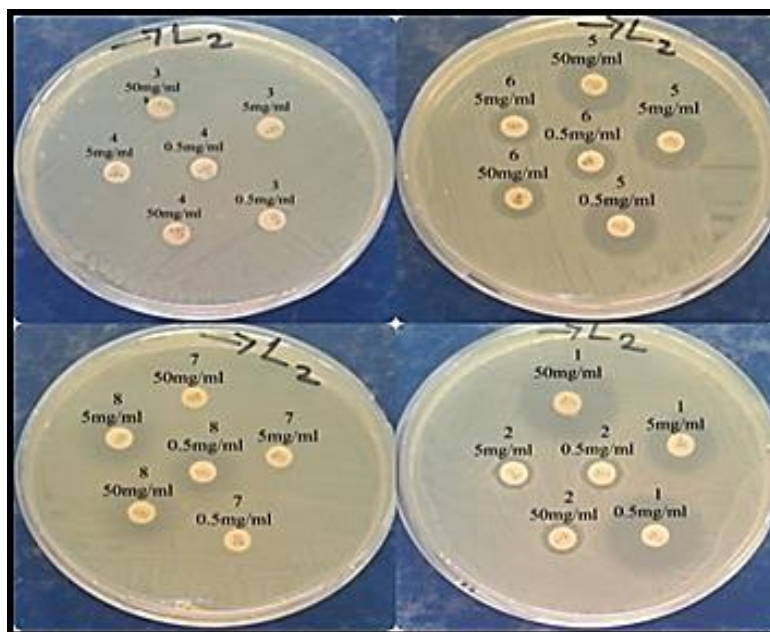
*M. luteus**S. aureus*

Figure 4. Synergistic and antagonistic effects of Azo-Schiff base with eight antibiotics against Gram-positive bacteria: 1= DA₂, 2=VA₅, 3=P₁₀, 4= AMP₁₀, 5= CN₁₀, 6= S₁₀, 7= MA₃₀, 8= C₁₀.

CONCLUSION

In an attempt to test the efficacy of Schiff base alone on three bacterial isolates, and with known antibiotic concentrations, we conclude the following:

- Schiff base have activity only on *S. aureus* at concentration of 50 mg/ml.
- The synergistic effect of Schiff base was shown with antibiotic CN₁₀.
- The antagonistic effect was observed with all the antibiotics.

Although these results may benefit the field of pharmacology to some extent, but they are not sufficient on this Azo-Schiff base in high concentrations. We also need more studies on other Schiff base with these antibiotics to overcome the problem of resistance against antibiotics.

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