

Microbiological studies of a new naphthoquinone semicarbazone derivatives

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Abstract

The antimicrobial activity of two naphthoquinone semicarbazone derivatives (Two newly synthesized compounds) have been studied by using tube – dilution and disc plate technique. The effect of those derivatives upon pathogenic microorganism isolated from specimen (urine, wounds, stool, swabs, throatetc) have been studied also in comparison with the antibiotics (amikacin, ampicillin, carbencillin, cephalothin, cefoxitin, clindamycin, erythromycin, gentamycin, penicillin, tetracylin and trimethoprim. It was shown that derivative(1) had more effective against micro organism than derivative(11).

Introduction

Naphthoquinone compounds are one of the chemicals that have prepared previously owing to their biological activity such as antibacterial, anticarcinogenic and antioxidant agents⁽¹⁻⁶⁾ they are very potent inhibitor to the DNA biosynthesis through its interaction such as 2 – amino 1,4 – naphthoquinone imine⁽⁷⁾. Due to the importance of this class of compounds, a new semicarbazone derivatives have been prepared⁽⁸⁾, in the present investigation the antimicrobial activity of those new derivatives have been studied by estimating the ability of them as antimicrobial agents *in vitro*.

Materials and Methods

1- Chemical assay

The semicarbazone derivatives of 4 – amino – 1,2 naphthoquinone (I) and 2 – acetylamino – 1,4 naphthoquinone (II) have been prepared (scheme 1) and recrystallized.

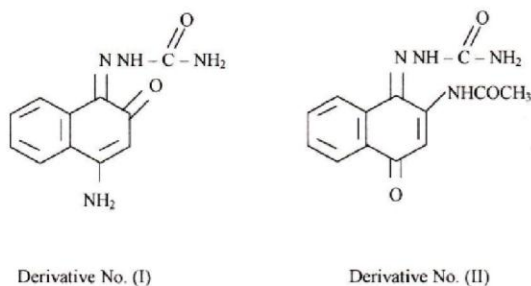
The two derivatives were insoluble in water and soluble in dioxane, dimethyl sulphoxide (DMSO) and acetic acid. A solution of those two derivative were prepared by dissolving them in (DMSO) to obtain final concentration 1000 mg/m.

2- Biological Assay

Biological potency was expressed in term of microorganism sensitivity to the two derivatives as antimicrobial agents, The methods which have been used were:

a- Tube – dilution, in this technique the minimum inhibitory concentration (MIC) of the derivatives to inhibit the growth of organism was determined.

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Scheme 1

b- Disc – plate technique, the disc diffusion method originally described by Kirby and association in (1966)⁽⁹⁾ has been followed in this investigation. A disk of each derivatives was prepared⁽¹⁰⁾ at a concentration of 200 Mg/ml. A commercially available discs with proper diameter and potency were used, including amikacin, ampicillin, carbencillin, cephalothin, cefoxitin, clindamycin, erythromycin, gentamycin, penicillin, tetracyclin and trimethoprim (unidisc oxid L.T.D). Mueller Hinton agar media was used (Difco Manual 1974, p-92). Stock organism obtained from central public health laboratory. *Escherichia coli* NCTC 10418, *pseudomonas aeruginosa* NCTC 10602.

Staphylococcus aureus NCTC 6571 and *candida albicans*. Standard antibiotic discs cephalothin (30 mg) for gram positive bacteria, amikacin for gram – negative bacteria were chosen in this investigation for testing the activity if these derivatives on the microorganism isolated from the specimen, The following specimen and microorganism isolated. Gram – positive bacteria

Streptococci spp 10 were isolated form urine

Haemophilis spp 10 were isolated from vagina

Staphaureus spp 10 were isolated from wound

Gram negative bacteria

Escherichia coli spp 10 were isolated from stool

Pseudomonas spp 10 were isolated from stool

Proteus spp 10 were isolated from stool

Mueller – Hinton agar plate was evenly covered with a pure standardized inoculum of organisms used in this study.

The antibiotic discs and the prepared discs of synthesized derivatives were carefully placed up on them, after over night incubation, the zone diameter of inhibition around each disc is measured and interpreted as “sensitive” (or “susceptible”), “intermediate”, or “resistant” according to a zone – size interpretative chart⁽¹¹⁾.

Results and Discussion

The antimicrobial activity of the two naphthoquinone semicarbazone derivatives (newly synthesized compounds) have been measured as growth rate of pathogen isolated from specimen. Measurment were done against reference micro – organisms obtained from reference laboratory (public health laboratories). (table 1) shows the semicarbazone derivative I) ffect 90% of *staphaureous* and *Haemophilis* spp and affect 80% of *streptococci*, however the semicarbazone derivative II affect 70% of *staph – aureus* and *Haemophilis*, but it affecte 60% of *streptococci*. In comparison with antibiotics used it was found that cephalothin affect 100% of *streptococci*, *staph – aureus* and *Haemophilis* tetracyclin shows 100% sensitive of *Haemopilis* and 90% of *streptococci* and *staphaureous*, erythromycin affect 100% of *Haemophilis*, 80% of *streptococci* and 70% of *staphaureus*, Penicillin affect 100% of *Haemophilis*, 70% of *streptococci* and *staph – aureous* resistance to penicillin. Two organism shows resistance to

methoprim. *Staphaureus* and *Haemophilis*, while *streptococci* 60% sensitive. Semicarbazone derivative (I) shows highly sensitive of Gram – negative bacteria, it effect on *Escherichia coli* and *proteus* 100%, while *Pseudomonas* 90% sensitive, when compared with routin antibiotic such as Amikasin and Cephatoxim give 100% sensitive on *Escherichia coli*, *pseudomonas spp* and *proteus spp* respectively. Carbincillin affect 90% of all testing micro organism. Trimethoprim affect 90% of *E. Coli* and *proteus* respectively and 80% affect of *pseudomonas*, on the other hand *E-Coli* and *pseudomonas* shows resistance to Ampicillin and Tetracyclin. Semicarbazone derivative II had no affect upon the gram – negative bacteria *E. coli*, *pseudomonus spp* and *proteus spp* gave 100% resistance.

From the results obtained from the pilot study it shows that cephalothin affect 100% on gram – positive strain and amikacin gave 100% sensitive on gram – negative bacteria. So we chose these antibiotics as standard drug to be used with semicarbazone derivatives (I) and (II) as shown on table (2) which demonstrate the antimicrobial activity of derivatives (I) & (II) on gram – positive and gram – negative reference bacteria compared with standard antibiotics amikacin and cephalothin. It was shown in this table the zone diameter of standard drug compared with the zone diameter of antimicrobial activity of the two naphthoquinone semicarbazone derivatives.

It was found that derivative (I) gave equal or the same zone diameter which shown by Amikacin 18mm sensitive, 17mm intermediate and 14 mm resistance, while the derivative (II) shows resistance on gram – negative bacteria. In the same table we notice that derivative (I) shows nearly the same diameter as given by cephalothin on *staph aureus*, 17mm sensitive, 15mm

intermediate, and 14mm resistance, while the derivative (II) shows nearly moderate sensitive or resistance. *Candida albicans* shows sensitivity to the derivative (I) (moderately) with diameter 8mm and derivative (II) shows 6mm diameter.

In conclusion, from the results obtained in this study it was shown that the derivative (I) was more effective against micro-organism than derivative (II) and have been particularly suited for use as adisinfactant in soaps, creams or oil applied to the skin for reducing and controlling the microbial flora. Future studies will be conducted to see the safety of using this staff as amedicin in vivo.

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Table (1) Antimicrobial sensitivity test of the two semicarbazone according to antimicrobial susceptibility patterns

Organism	No. of strain	Percentage susceptibility to:								
		Pen.	Meth.	Eryt.	Cepha	Tetra	Clin	Derivatives		
Gram (+ve) cocci	30							I	II	
Streptococci	10	70	60	80	100	90	80	80	80	
Staph - aureus	10	R	R	70	100	90	80	90	70	
Haemophilis	10	100	R	100	100	100	80	90	90	
Gram (-ve) Bacilli	30	Cefa.	Tetr.	Amp.	Carb.	Gent.	Trim.	Amik.	derivatives	
									I	I
Escherichia coli	10	100	R	R	90	70	90	100	100	R
Pseudomonas	10	100	R	R	90	80	80	100	90	R
Proteus	10	100	60	90	90	70	90	100	100	r

Note: Pen: Penicillin; Meth: Methoprine; Eryt: Erythromycin; Cepha: Cephalothin; Tetr: Tetracyclin;

Clin: Clindamycin; Cefa: Cefatoxim; Amp: Ampicillin; Carb: Carbincillin; Gent: Gentamycin; Amik: Amikacin.

Table (2) Antimicrobial activity of semicarbazone derivatives (I) and (II) on gram positive and gram negative reference bacteria compared with standard antibiotics Amikacin and Cephalothin

Organisms	Gram (- ve) Bacilli					
	E. Coli			Pseudomonas - areogenosa		
	Zone diameter (mm)					
Antimicrobial	S	In	R	S	In	R
Standard drug Amikacin	18	17	15	18	16	14
Derivative (I)	18	17	14	17	16	14
Derivative (II)	-	-	-	-	-	-
Organism	Gram (+ ve) cocci					
	Staph - aureus			Candida - albicans		
	S	In	R	S	In	R
Standard drug Cephalothin	18	16	14	-	-	-
Derivative I	17	15	14	8	6	5
Derivative II	9	8	7	6	5	5

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دراسة الفعالية المايكروبايولوجية لاثنين من مشتقات النافافثوكوينون النصف كاربازونية الجديدة

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الخلاصة

تم دراسة الفعالية المايكروبايولوجية لاثنين من مشتقات النافثوكيتون النصف كاربازونية (المحضرة حديثاً) باستخدام الطرائق الحياتية. كذلك تم دراسة تأثير هذه المشتقات على مايكروبات مرضية تم عزلها من نماذج مختلفة (الادار، الجروح، الخروح، المسحات، اللوزتين الخ) وبالمقارنة مع المضادات الحيوية (الاميكاسين، الامبسلين، الكاربنسولين، السيفالوثين، السيفوكستين، الكلنداميسين، الارثرومايسين، الـ جنتاميسين، البنسلين، التتراسايكلين، والتراي مثيريم...) لقد وجد بان المشتق (I) كان اكثر فعالية من المشتق (II) تجاه المايكرو المرضية.