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Study of microbial Activity of Some Arylorganomercury (II) Derivatives Containing Amino Group on Some Pathogenic Isolated Bacteria

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ABSTRACT

The aim of this study was to evaluate a microbial activity of the three organomercury compounds :2-amino-5-methylphenyl mercury(II)chloride 1,2-amino-5-bromophenyl mercury(II)chloride 2 and 2-amino-5-nitrophenyl mercury(II)chloride 3 which were synthesized and characterized by various physical techniques . It have been reported to have antimicrobial activity against six various bacteria such as Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram negative bacteria (Escherichia coli , Klebsiella spp., Salmonella spp. and Pseudomonas spp.) . The concentrations of above compounds 1,2 and 3 were (0.5 , 0.3–0.5 and 0.3–0.5 milligram / milliliter) found to be more effective concentrations than others related with the inhibition zone respectively . The order of antimicrobial activity for the studied compounds were depending on the power of the drawing group with mercury moiety and amino group abreast in the aromatic skeleton – structure . So , the order is NO 2 (3) > Br (2) > CH 3 (1)

Introduction

Microbial resistance of a pathogenic bacteria is a continuing worldwide issue and as a consequence , effective treatment and control of such organisms remains an important challenge . Bacterial resistance has appeared for every major classes of antibiotics [1] . Since their introduction the emergence of resistance to antibiotics has become increasingly evident, particularly for important pathogens such as Escherichia coli and Salmonella spp. [2 – 3] . Many bacteria have advanced protective mechanisms for the detoxification of heavy metal ions [4] . Despite this , numerous literature reports address the development of metal compounds as antimicrobial agents [5] . Many low molecular mass metal compounds exhibit bactericidal and / or bacteriostatic activities [6] . New metalloantibiotic agents include a range of ligands that have been chelated to metal ions and to date, antimicrobial activites have been demonstrated for metal complexes of imidazoles , phenanthrolines, quinolones , aminoquinolines and benzoylhydrazones [5–10] . A wide range of arylmercury (II) compounds were prepared and investigated for many purposes [11 - 12] .

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Materials and methods

Materials and physical measurements :

Dimethylsulphoxide (DMSO) solvent was dry and product of Fluka company . The compounds 2-amino-5-methylphenyl mercury(II)chloride 1,2-amino-5-bromophenyl mercury(II)chloride 2 and 2-amino-5-nitrophenyl mercury(II)chloride 3 were prepared as described in the literature [12] , fitted by their melting points and infrared spectra . Infrared spectra were recorded in the range 400 – 4000 cm -1 using a Brucker IFS-113 , KBr disc . Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected .

Inoculation of plates :

It was done by the method as described in the literature [13] . The six various bacteria have been taken from laboratories of department of Biology , College of Science , University of Baghdad . Bacterial suspension was compared with tube number 0.5 of McFarland which equal to (1.5 x 10 8 CFU / ml) was freshly prepared and 2 ml of this suspension was transferred to Muellar Hinton Agar plate . The excess fluid was removed from the plates which was kept in incubator at 37 0C for (24 hrs) to dry before the

application of discs . The five discs were distributed gently over the surface of medium with rocking . Isolates were re-identified and confirmed by carrying out Gram staining procedures , lactose fermenter on MacConkey agar medium and IMVIC test [14 - 15] . The cultures of bacteria were maintained in their appropriate agar slant at 4 0C through out the study and used as stock cultures .

Susceptibility test using disc diffusion method :

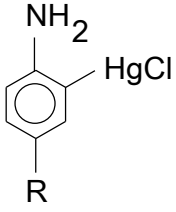
It was carried out by using the method as described in the literature [14] . Each of the mentioned compounds 1 , 2 and 3 (scheme 1) were dissolved in dry DMSO solvent and solutions of (0.1 , 0.2 , 0.3 , 0.4 , 0.5 milligram / milliliter) concentrations were prepared . Thus , stock solution was prepared by dissolving 0.1 gm of each compound in 10 ml solvent , then prepare the series of concentrations by transferring (0.1 , 0.2 , 0.3 , 0.4 , 0.5 ml) of stock solution into (9.9 , 9.8 , 9.7 , 9.6 , 9.5 ml) of distilled water respectively . Total volume becomes 10 ml for each concentration . During sensitivity testing , a drop of diluted form was kept on filter paper disc of 6 mm diameter , placed on bacterial suspension , then the zone of inhibition were measured in millimeter .

Sterilization of material :

The media , paper disc (which was in aluminum foil) , glass apparatus and micropipette were autoclaved at 121 0C for 15 minutes under pressure 15 atmosphere [7].

Results and discussion

The synthesis , characterization and antimicrobial activity of some organomercury compounds containing amino group of the type (2-NH2-5-R-C6H3-HgCl) , where [R = CH3 (1) , Br (2) , NO2 (3)] were reported (scheme 1) .



R = CH_3(1), Br (2), NO (3) 2

2-Amino-5-methylphenyl mercury(II)chloride 1
2-Amino-5-bromophenyl mercury(II)chloride 2
2-Amino-5-nitrophenyl mercury(II)chloride 3
Scheme 1. The prepared compounds

When an equimolar mixture of aniline derivatives, mercury(II)acetate and lithium chloride were refluxed in dry methanol for 12 hours , gave the final products 1, 2 and 3 in 80% , 82% and 76% yields respectively . In this study , the three compounds have been reported to have a microbial activity against a range of Gram positive and negative bacteria at various concentrations. The results showed that the all compounds display a certain biological activity to Gram positive bacteria like (Staphylococcus aureus and Bacillus subtilis) and Gram negative bacteria like (Escherichia coli , Klebsiella spp., Salmonella spp. and Pseudomonas spp.). The data are summarized in tables 1 – 4), fifteen experiments (five various concentrations / each bacteria x three compounds) were tested at five concentrations (0.1– 0.5 mg / ml), (tables1–3). The inhibition zones have been shown different resistance pattern to various bacteria . The screening assay demonstrated that the all compounds that exhibited activity against the two types of organisms at 24 hrs in the test panel . In the compound 3 (table 3) , for the Gram positive organisms, the inhibition zones / active concentrations (7 mm / 0.1 mg / ml – 14 mm / 0.3 mg / ml) were observed to Staphylococcus aureus , while the inhibition zone / active concentrations (9 mm / 0.2 mg / ml – 10 mm / 0.3 mg / ml) were detected to Bacillus subtilis . This may indicate an inhibition of physiological processes that is overcome upon extended incubation . For the Gram negative organisms (compound 3 , table 3), it showed a biological activity against Escherichia coli , Klebsiella spp., Salmonella spp. and Pseudomonas spp. bacteria .

The inhibition zones / active concentrations (7 mm / 0.1 mg / ml – 10 mm / 0.4 mg / ml) , (8 mm / 0.2 mg / ml – 10 mm / 0.4 mg / ml) , (8 mm / 0.5 mg / ml – 11 mm / 0.4 mg / ml) and (8 mm / 0.2 mg / ml – 10 mm / 0.4 mg / ml) were detected respectively . In the compounds 1 and 2 , the tests and the calculations were done analogous carefully (tables 1–2) . Out of thirty tests against six isolates at five various concentrations (tables 1 - 3) , only ten , twenty four and twenty six of total - sensitive concentrations were inhibited by the 2-amino-5-methylphenyl mercury(II)chloride 1,2-amino-5-bromophenylmercury(II)chloride 2 and 2-amino-5-nitrophenyl mercury(II)chloride 3 respectively .

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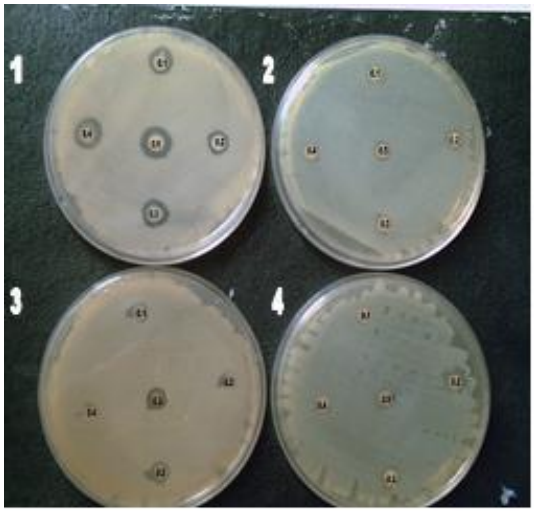

Figure 1. Effect of 2- amino-5-nitrophenylmercury(ll)chloride 3 on the bacteria

1. S.aureus , 2. E.coli , 3. Bacillus subtilis , 4. Klebsiella Spp. respectively .

The current study and report demonstrate that antimicrobial properties of aminoaromatic derivatives can be enhanced by metals moiety like mercury in the skeleton – structure [10] . Generally , to the Gram negative bacteria , the all studied compounds resulted in no detectable growth for the all isolates at active concentrations and giving better results more than Gram positive bacteria (tables 1 – 3) .

Table 1 . A diameter of inhibition zone by the effect of of the various concentrations for 2-amino-5-methylphenyl mercury(II)chloride 1 on the various bacterial species .

No.	* Bacterial species	A diameter of inhibition zone in (millimeter) Concentrations in (milligram / milliliter)					Solvent DMSO
		0.1	0.2	0.3	0.4	0.5	
1	*aureus.Staph*	10	8	9	10	13	-
2	*Bacillus subtilis*	-	-	-	8	8	-
3	*Escherichia coli*	-	-	-	-	8	-
4	*Klebsiella spp.*	-	-	-	-	-	-
5	*Salmonella spp.*	-	-	-	-	7	-
6	*Pseudomonas spp.*	-	-	-	-	7	-

**** Staphylococcus aureus and Bacillus subtilis were G $^{+}$Ve bacteria , Escherichia coli , Klebsiella spp. , Salmonella spp. and Pseudomonas spp. were G $^{-}$Ve bacteria .***

Table 2 . A diameter of inhibition zone by the effect of the various concentrations for 2-amino-5-bromophenyl mercury(II)chloride 2 on the various bacterial species .

No.	Bacterial species	A diameter of inhibition zone in (millimeter) Concentrations in (milligram / milliliter)					ent DMS
		0.1	0.2	0.3	0.4	0.5	
1	*Staphylococcus aureus*	7	-	9	12	7	-
2	*Bacillus subtilis*	-	7	8	10	8	-
3	*Escherichia coli*	7	7	8	13	8	-
4	*Klebsiella spp.*	-	7	8	12	8	-
5	*Salmonella spp.*	-	7	10	10	8	-
6	*Pseudomonas spp.*	-	-	9	10	8	-

Table 3 . A diameter of inhibition zone by the effect of the various concentrations for 2-amino-5-nitrophenyl mercury(II)chloride 3 on the various bacterial species .

No.	Bacterial species	A diameter of inhibition zone in (millimeter) Concentrations in (milligram / milliliter)					Solvent DMSO
		0.1	0.2	0.3	0.4	0.5	
1	*Staph. aureus*	7	13	14	13	8	-
2	*Bacillus subtilis*	-	9	10	10	11	-
3	*Escherichia coli*	7	8	9	10	9	-
4	*Klebsiella spp.*	-	8	9	10	10	-
5	*Salmonella spp.*	-	9	10	11	8	-
6	*Pseudomonas spp.*	-	8	9	10	8	-

The screening results showed a compound 2-amino-5-nitrophenyl mercury(II)chloride 3 has more a microbial activity than the others . Thus , a compound with drawing groups like nitro type is more effective than a compound with releasing groups like methyl type and a bromo compound is the border between them . So , the order of power - microbial activity for the studied compounds are :

NO 2 (3) > Br (2) > CH 3 (1)

It may be attributed that the presence of mercury moiety with strong – drawing group and amino group abreast would give a good biological activity in aromatic – skeleton system . Generally , For the all isolates , the active concentrations were (0.5 , 0.4 and 0.4 mg / ml) to the compounds 1 , 2 and 3 respectively . Overall , according to the statistical data (table 4) , Out of ninety tests (15 tests / each against six isolates) , only sixty of the total no. of sensitive concentrations were inhibited by the studied compounds .

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Table 4 . A statistical data and a diameter of inhibition zone by the effect of the various concentrations for the compounds 1 , 2 and 3 on the various bacterial species .

The various concentra-tions of the compoun-ds 1 , 2 and 3	Terms and tests	A statistical data and a diameter of inhibition zones					
		Bacterial species					
		Staph-yloco-ccus aureus	*Bacil-lus subtil-is*	*Escher-ichia coli*	*Klebs-iella spp.*	*Salmo-nella spp.*	*Pseud-omonas spp.*
	Total no. of tests -each isolate / each comp.	15	15	15	15	15	15
	Total no. of sensitive conc. / each isolate.	14	10	11	8	9	8
0.1 mg / ml of comp. 1	**Inhibit ion zone in (mm)	10	-	-	-	-	-
0.1 mg / ml of comp. 2	=	7	-	7	-	-	-
0.1 mg / ml of comp. 3	=	7	-	7	-	-	-
0.1 mg / ml of comp. 1	No. of sensitive con.***	1	0	0	0	0	0
0.1 mg / ml of comp. 2	=	1	0	1	0	0	0
0.1 mg / ml of comp. 3	=	1	0	1	0	0	0
0.2 mg / ml of comp. 1	Inhibitio n zone	8	-	-	-	-	-
0.2 mg / ml of comp. 2	=	-	7	7	7	7	0
0.2 mg / ml of comp. 3	=	13	9	8	8	9	8
0.2 mg / ml of comp. 1	No. of sensitive con.	1	0	0	0	0	0
0.2 mg / ml of comp. 2	=	0	1	1	1	1	0
0.2 mg / ml of comp. 3	=	1	1	1	1	1	1
0.3 mg / ml of comp. 1	Inhibitio n zone	9	-	-	-	-	-
0.3 mg / ml of comp. 2	=	9	8	8	8	10	9
0.3 mg / ml of comp. 3	=	14	10	9	9	10	9
0.3 mg / ml of comp. 1	No. of sensitive con	1	0	0	0	0	0

Table 5 :The various concentra-tions of the compoun-ds 1 , 2 and 3

The various concentra-tions of the compoun-ds 1 , 2 and 3	Terms and tests	A statistical data and a diameter of inhibition zones					
		Bacterial species					
		Staph-yloco-ccus aureus	*Bacil-lus subtil-is*	*Escher-ichia coli*	*Klebs-iella spp.*	*Salmo-nella spp.*	*Pseud-omonas spp.*
	Total no. of tests -each isolate / each comp.	15	15	15	15	15	15
	Total no. of sensitive conc. / each isolate	14	10	11	8	9	8
0.3 mg / ml of comp. 2	No. of sensitive concentra tion	1	1	1	1	1	1
0.3 mg / ml of comp.3	=	1	1	1	1	1	1
0.4 mg / ml of comp. 1	Inhibition zone	10	8	-	-	-	-
0.4 mg / ml of comp. 2	=	12	10	13	12	10	10
0.4 mg / ml of comp. 3	=	13	10	10	10	11	10
0.4 mg / ml of comp. 1	No. of sensitive concentra tion	1	1	0	1	0	0
0.4 mg / ml of comp. 2	=	1	1	1	1	1	1
0.4 mg / ml of comp. 3	=	1	1	1	1	1	1
0.5 mg / ml of comp. 1	Inhibition zone	13	8	8	-	7	7
0.5 mg / ml of comp. 2	=	7	8	8	8	8	8
0.5 mg / ml of comp. 3	=	8	11	9	10	8	8
0.5 mg / ml of comp. 1	No. of sensitive concentra tion	1	1	1	0	1	1
0.5 mg / ml of comp. 2	=	1	1	1	1	1	1
0.5 mg / ml of comp. 3	=	1	1	1	1	1	1

** (--) : It means no effect and no inhibition zone accordingly .
*** No. 1 : it means , there is amicrobial activity , while No. 0 = there isn't .
Comp. 1 : 2-Amino-5-methylphenyl mercury(II)chloride .
Comp. 2 : 2-Amino-5-bromophenyl mercury(II)chloride .
Comp. 3 : 2-Amino-5-nitrophenyl mercury(II)chloride .

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دراسة الفعالية المايك روبية لبعض مشتقات الزئبق الثنائية الاروماتية الحاوية مجموعة أمين على بعض العزلات البكتيرية المرضية

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الخلاصة:

الهدف من الدراسة – تحديد وتعيين الفعالية المايكروبية لثلاثة مركبات من بعض مشتقات الزئبق الثنائية الاروماتية – الحاوية على مجموعة أمين. لقد تم تحضير مركبات (٢– أمينو – ٥ – معوّض – فينيل كلوريد الزئبق الثنائي)، عندما يكون [المعوّض = مجموعة مثيل (١)، مجموعة برومو (٢)، مجموعة نيترو (٣)] والتي تمّ تشخيصها بوساطة مختلف التقنيات الفيزيائية المتعارف عليها. وقد أظهرتْ الدراسة المايكروبية فعالية ونشاط لهذه المركبات ضد مختلف أصناف البكتيريا الموجبة والسالبة مثل: Staphylococcus aureus , Bacillus subtilis Escherichia coli , Klebsiella spp. , Salmonella spp. , Pseudomonas spp.

أظهرتْ التراكيز الفعالة (٠.٥ ، ٠.٣ – ٠.٥ ، ٠.٣ – ٠.٥ مليغرام / مليليتر) للمركبات (١)، (٢)، (٣) على التوالي تأثيرا" محسوسا" أكثر من التراكيز الاخرى في تثبيط عمل البكتريا الموجبة والسالبة لست عزلات بكتيرية مرضية – موضوع هذه الدراسة . دلتّ النتائج على أن قوة الفعالية المايكروبية للمركبات الثلاثة تتدرج حسب قوة المجموعة الساحبة للالكترونات بوجود ذرة الزئبق ومجموعة الامين جنبا" الى جنب في النظام الاروماتي وكما يأتي: –مركب النيترو (٣)> مركب البرومو (٢)> مركب المثيل(١).