Biological activity of 4-(2-methoxy benzylidene amino) phenyl mercuric chloride and 4-(2-chlorobenzylidene amino) phenyl mercuric chloride compounds

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Abstract

The 4-(2-methoxy benzylidene amino) phenyl mercuric chloride and 4-(2-chlorobenzylidene amino) phenyl mercuric chloride were investigated for their biological activity against standard strains of *Staphylococcus aureus* ATCC 25923 and *Ecsherichia coli* ATCC 25922. The results showed that there is a potent antibacterial activity of these compounds. Minimal inhibitory concentration was determined for these two compounds, it was 4 µg/ml, 5 µg/ml for *Staph. aureus*, and 15 µg/ml, 20 µg/ml for *E. coli* respectively. Cytotoxicity assay was carried out against human red blood corpuscles, the two compounds exhibited a toxic effect in high concentrations on RBCs while in high diluted concentrations have no toxic effect on it.

Introduction

Medical practices have evolved overtime, with the raise of technology and more advanced techniques, new methods of treating diseases and maintaining good health have been introduced. In order to develop these new methods, certain important elements had been utilized, inorganic compounds of various elements have been useful for medical purposes (The Columbia Encyclopedia, 2004) , mercurials is one of the most common compounds related to medical use. Mercury is a naturally occurring metal due to erosion from earth crusts and volcanoes (Clarkson, 2002), exist naturally in the environment in three major forms. These include the metallic, inorganic and organic mercury (World Health Organization , 2003). A number of inorganic mercury compounds had been used as fungicides, like methyl mercury, which had been used for protection from fungal infections. Disinfectant agents or topical antiseptics are also one of the medicinal products that use inorganic mercury, specifically mercuric chloride. Antibacterial medicines also include inorganic mercury which is used in these medications because of its preservative action for certain medications and over the counter drugs (Chatterjee, 2002), thimerosal has been used as preservative in medical preparations including vaccines (Magos, 2001). Different mercury salts used for cutaneous applications that is used for treating an infected impetigo or eczema or psoriases (Brown, 2003). While mercury and its inorganic forms had been used for pharmaceutical uses, mercury has been useful for important medical apparatuses including thermometers, blood

pressure monitors, esophageal dilators and feeding tubes (Mahaffy , 1999).

Aim of this investigation*:* In accordance with the wide uses of mercury and the powerful effect of mercuric compounds against bacteria, the aim of this investigation was to use these compounds as disinfectants for surgical instruments as an essential part of infection control practices.

Materials and methods

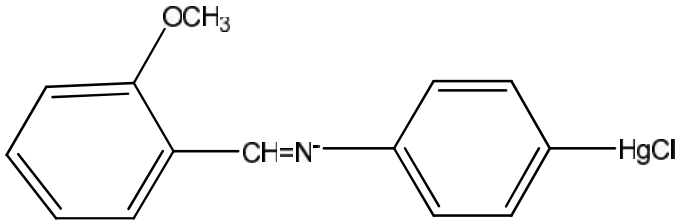
Bacterial isolates

Two standard strains of *Staph. aureus* ATCC 25923 and *E. coli* ATCC 25922 were obtained from the Research laboratory in Pharmacy College which have been used in this investigation.

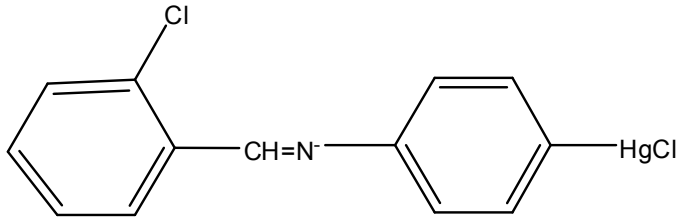
Synthesis:

General Procedure

The two compounds used in this investigation were prepared and identified according to the literature of (Nebmeyanov and Kocheshov, 1967) and characterrized by elemental analysis that gave good results to confirm the below structures. A mixture of 4-aminophenyl mercuric chloride (2.43 g, 8.00 mmol.) in 50 ml of ethanol and an aldehyde *i.e* 2methoxybenzaldehyde or 2-chlorobenzaldehyde) (8.00 mmol. in 50 ml of ethanol containing 0.1 g of p-Toluen sulfonic acid were reflexed with stirring for 5 hrs. after cooling, then the precipitate was collected by filtration and washed several times with ethanol. The solid product re-crystallized twice from a mixture of ethanol and benzene (3:2) to give yellow solids for both compounds, yields 92% and 81% respectively, melting points 163-165°C and 160-162°C respectively.



4-(2-methoxy benzylidene amino) phenyl mercuric chloride



4-(2-chloro benzylidene amino) phenyl mercuric chloride

Antibacterial activity

To make first screening of the activity for the 4-(2-methoxy benzylidene amino) phenyl mercuric chloride and 4-(2-chlorobenzylidene amino) phenyl mercuric chloride, the *in vitro* antibacterial activity was tested against two standard strains of *Staph. aureus* and *E. coli*, by using disc diffusion method **(**Collee *et al .,* 1996 **)**. Nutrient agar plates were inoculated with 24 hrs. growth (containing 10^6 CFU/ml, according to McFarland standard scale) of both two standard strains (McFarland, 1907), after that discs that are impregnated with two mercuric compounds in different concentrations ranged between 1000 µg/ml to 1 µg/ml were placed on inoculated nutrient agar plates and incubated at 37ºC for 24 hrs., then the inhibition zones for each concentration were measured. The experiment was repeated three times under the same conditions for each compound.

Minimal inhibitory concentrations (MICs)

By using broth micro dilution

method **(**NCCLS, 2003**)**, MICs were detected for both two mercuric compounds under investigation. Nutrient broth test tubes that contain different concentrations of mercuric compounds that ranged between 4µg/ml to 1000µg/ml which were inoculated with 24hrs. culture of standard strains of *Staph. aureus* and *E. coli*, then incubated at 37ºC for 24 hrs., the results were examined visually and by re-cultivation at the same conditions of MIC test tubes contents that poured on nutrient agar plates, control plates were carried out for each bacterial isolate without adding the mercuric compounds.

Cytotoxicity

The cytotoxicity of 4-(2-methoxy benzylidene amino) phenyl mercuric chloride and 4-(2-chlorobenzylidene amino) phenyl mercuric chloride was assayed against human red blood corpuscles (RBCs) according to **(**Nair *et al*., 1989**)**. 2 ml of human RBCs (with EDTA as anti-coagulant) were mixed with 38 ml of Ringer solution, the mixture dispensed in 2 ml dry, clean test tubes. Different concentrations of mercuric compounds were prepared (1, 10, 30, 50, 100, 200, 300, 400, 500 µg/ml) respectively, after that added to the suspension of RBCs and Ringer solution, and incubated for 8 hrs. at 37ºC, then the results were recorded.

Results and Discussion

Elemental analysis

The results of elemental analysis of the studied compounds are shown in table (1).

Table (1). Elemental analysis of two mercuric compounds

Compounds		C (cal.)	H (cal.)	N (cal.)
1.	4-(2-methoxy benzylidene amino) phenyl mercuric chloride	37.53 37.68	2.58 2.71	3.18 3.14
2.	4-(2-chloro benzylidene amino) phenyl mercuric chloride	34.38 34.64	2.06 2.01	3.15 3.11

Cal. = calculated

Antibacterial activity

The results of first screening by disc diffusion method exhibited a potent antibacterial activity for mercuric compounds under investigation against the tested bacteria, represented by inhibition zones diameters recorded after bacterial growth table (2).

Table (2). Antibacterial activity of two mercuric compounds by Disc Diffusion Method

Compounds		Standard strains	Inhibition zone diameters (mm)
1.	4-(2-methoxy benzylidene amino) phenyl mercuric chloride	*Staph. aureus*	37
		E. coli	30
2.	4-(2-chlorobenzylidene amino) phenyl mercuric chloride	*Staph. aureus*	32
		E. coli	29

mm = millimeter

These results considered the first step to assess the activity of two compounds and confirmed antibacterial activity of both, also showed differences in the inhibition zones diameters between G +ve (Gram positive)and G –ve (Gram negative) bacteria, since *Staph. aureus* was more sensitive to the action of these compounds which were indicated by wide, clear inhibition zones in comparison with *E. coli*, and this may be ascribed to the differences in the nature of the cell wall components of G +ve and G –ve bacteria.

The G –ve cell wall is structurally more complex than the G +ve cell wall, comprised of peptidoglycan biopolymers, but there are only a few layers compared to several layers observed in G +ve bacteria, and they possess an inner and outer cell membrane. The amino acid side chains of the peptidoglycan subunits are not connected by peptide inter bridges but are directly covalently bonded to each other and are suspended in the periplasmic space, an area between the cell membranes, extending beyond the peptidoglycan is an outer cell membrane, comprised of a phospholipid bi-layer, with an outermost zone comprised of chemically complex lipopolysaccharides (LPS), that serve in permeability characteristics (Nelson *et al*., 2009). The outer membrane limits molecular transfer and permeation with embedded specialized porins that may act as permeability barriers that allow selective passage of nutrients and exclusion of harmful substances e.g. antimicrobial agents, also allow entry of substrates and other small molecules, chemotactic factors and also antibiotics (Todar, 2004). While in the G +ve bacteria, cell wall is characterized by the presence of a very thick, loose peptidoglycan layers without intact cross links, and without

outer membrane outside the peptidoglycan layers, this makes G +ve bacteria cell wall structurally weak and more susceptible to antibiotics and other antimicrobial agents (Vreeland *et al*., 2000).

Minimal inhibitory concentrations

The minimal inhibitory concentration values of 4-(2-methoxy benzylidene amino) phenyl mercuric chloride equaled 4 µg/ml, 15 µg/ml for standard strains of *Staph. aureus* and *E. coli* respectively and 5 µg/ml, 20 µg/ml for the 4-(2-chlorobenzylidene amino) phenyl mercuric chloride, the data are presented in table (3).

Table (3). MICs values applied on standard strains of *Staph. aureus* and *E. coli*

Compounds		Standard strains	MIC (µg/ml)
1.	4-(2-methoxy benzylidene amino) phenyl mercuric chloride	*Staph. aureus*	4
		E. coli	15
2.	4-(2-chlorobenzylidene amino) phenyl mercuric chloride	*Staph. aureus*	5
		E. coli	20

mm = millimeter, µg/ml = microgram/milliliter

The results showed the high activity of both two mercuric compounds in low concentrations confirmed by the re-cultivation of MICs test tubes contents on nutrient agar plates which showed

slight growth on these plates in comparison with control plates which exhibited a heavy bacterial growth. The mechanism of antimicrobial action of these two compounds were not known, but one can suggest that the two compounds at bactericidal level disrupt cell metabolism by binding through hydrogen bond with amino acids and proteins including enzymes or by the coordination of amino acids and proteins including enzymes with mercury atom through available vacant orbital, or might cause destruction of the bacterial cell wall(Rosa *et al*., 2005).

Mercury has a very important biochemical property, is the strong affinity to sulfhydryl groups (thiols), which leads to the formation of complexes called mercaptides. Sulfhydryls are specially common in cysteine-rich proteins, and bringing of mercury to thiols even at a low concentrations may have distinct effects on cell function (Wang and Horisberger, 1996).

Cytotoxicity

The results of cytotoxicity assay of 4-(2-methoxy benzylidene amino) phenyl mercuric chloride and4-(2-chlorobenzylidene amino) phenyl mercuric chloride against human RBCs revealed that these compounds have a toxic effect on RBCs in high concentrations, the microscopic examination under 40X power to the RBCs showed a haemolysis and destruction of the RBCs in these concentrations after incubation for 1 hour**,** but on the other side, these compounds in highly diluted concentrations overcome its toxicity and have no toxic effect on RBCs. The obtained results suggest that these compounds can be used as disinfectant in high diluted concentrations The dilute aqueous of organic mercurials are powerful antiseptics used for decontaminating skin

before surgical operations and for sterilizing surgical instruments (Rosette, 2002).

Wells, confirmed that mercuric chloride is sometimes used in dilute solutions as disinfectant for inanimate objects and as fungicide **(**Wells, 1984**)**.

Recommendations

We recommended to use high diluted solutions of these compounds which have no toxic effect and can be used as disinfectant for instruments used in surgery after an additional studies and experiments that required to prove the efficacy of these compounds.

Acknowledgment

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References

Brown, R. H. (2003). Mercury's fall from medicine to toxin. Georgia Public Policy Foundation.

Chatterjee, S. ; Pillai, A. and Gupta V. K. (2002). Spectrophotometric determination of mercury in environmental samples and fungicides based on its complex with O-carboxy phenyl diazoamino P-azobenzene. Talanta J. (57): 461–465.

Clarkson, T. W. (2002). The three modern faces of mercury. Enveron Health Perspect. 110 (suppl. 1) : 11-23.

Collee, J. G.; **Fraser, A. G.; Marmion, B. P. and Simon, A.** (1996). Mackie and McCartney Practical Medical Microbiology. 14th ed. Churchill Livingstone. New York. pp. 978.

Magos, L. (2001). Review on the toxicity of ethyl mercury including its presence as a preservative in biological and pharmaceutical products. J. Appl. Toxicol. 21:1-5.

Mahaffy, K. (1999). Methyl mercury a new look at the risks.

Public Health Reports. 114(5): 396.

McFarland, J. (1907). The Nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic Index and for Vaccines. J. Amer. Med. Assoc., 49:1176. Cited by : Practical Immunology. Hudson, L. and Hay, F. C. (1989).3rd ed. Blackwell Scientific Publications. Oxford p. 1-496.

Nair, M. G.; Putnam, A. R.; Mishra, S. K.; Mulks, M. H.; Taft, W. H.; Keller, J. E.; Keller, J. R.; Zhn, P. P.; Meinhart, J. D. and Lynn, D. G.(1989). Faeriefungin: A new broad spectrum antibiotic from *Streptomyces griseus* var *autotrophicus* J. Nat. Prod. 52(4):797-809.

NCCLS- National Committee for Clinical Laboratory Standards (2003). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard, ed 6. Wayne.

Nebmeyanov, A. N. and Kocheshov, A.V. (1967). Methods of Elements Organic Chemistry.1sted Northland Publishing Company Amsterdam.

Nelson, M. L.; Mark, C. G.; Susan, E. B.; and Mohamed Y. I. (2009). Polyfunctional Antibiotics Affecting Bacterial Membrane Dynamics. Anti-Infective Agents in Medicinal Chemistry. (8): 3-16.

Rosa, M. C.; Angeles Garcia, M.; Lopez, C. and Elguero, J. (2005). The structure of 3,5 dimethylpyrazole/Carboxylic acids co-crystals. Arkivoc (vii): 91-101.

Rosette, R. M. (2002). Bioinorganic Chemistry, a short course. Willy - Inter science, p. 260.

The Columbia Encyclopedia. (2004). Inorganic chemistry 6th Edition. New York, Columbia University Press.

Todar, K. (2004). Review of Todar's Online Textbook of Bacteriology "The Good, the Bad, and the Deadly". Science Magazine, 304: 1421.

Vreeland, R. H.; Rosenzweig, W. D.; and Powers, D.W. (2000). Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature. 407:897-900.

Wang, X. and Horisberger, J. D. (1996). Mercury binding site on $Na^+/K^+(+)$ –ATPase : a cysteine in the first transmembrane segment. Molecul. Pharmacol. 50(3):687-691.

Wells, A. F. (1984). Structural Inorganic Chemistry, Oxford: Clarendon Press.

World Health Organization. (2003). Elemental mercury and inorganic mercury compounds: Human heath aspects, Geneva. 10-12 p.

الفعالية البايولوجية لمركبي 4- (2- ميثوكسي بنزايلدين امينو) فينايل كلوريد الزئبق و4- (2- كلورو بنزايلدين امينو) فينايل كلوريد الزئبق

أيمان علي سعيد

فرع العلوم السريرية المختبرية

كلية الصيدلة / جامعة البصرة

البصرة / العراق

الخلاصة

قدرت الفعالية البايولوجية لمركبي 4- (2- ميثوكسي بنزايلدين امينو) فينايل كلوريد الزئبق و4- (2- كلورو بنزايلدين امينو) فينايل كلوريد الزئبق ضد السلالات القياسية لجرثومتي المكورات العنقودية الذهبية والأشريكية القولونية وأظهرت النتائج الفعالية ضد الجرثومية العالية للمركبين. حدد التركيز المثبط الأدنى للمركبين وكان 4 مايكروغرام/مل و 5 مايكروغرام/مل لجرثومة المكورات العنقودية الذهبية وكان 15 مايكروغرام/مل و 20 مايكروغرام/مل لجرثومة الأشريكية القولونية على التوالي ولكلا المركبين. تم أختبار السمية الخلوية للمركبين المحضرين ضد كريات الدم الحمراء للأنسان، وأظهر المركبان تأثيراً ساماً على كريات الدم الحمراء في التراكيز العالية في حين لم يظهر المركبين اي تأثير سام في التراكيز الأقل و عالية التخفيف على كريات الدم الحمراء.