Biological Activity of The Complexes of Hg(II), Zn(II) and Cd(II) Mixed Ligands (Thiosemicarbazone and Azine) part II*

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Abstract

The biological activity of the two ligands (benzaldehydethiosemicarbazone-BTSCH and 3,4–dimethoxybenzaldazine- DMBA) and their zinc(II)-, cadmium(II)- and mercury(II)- complexes having the formula $M_2(BTSCH)_2(DMBA)_2X_4$ and M(BTSC)(DMBA)X (where BTSC=deprotonated BTSCH ligand; M = Zn^{2+}, Cd^{2+}, or Hg^{2+}; X = NO_3^- or Cl^- or I^-) in dimethylsulphoxide solution have been evaluated by agar plate diffution technique against five human pathogenic bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsella pneumonia*, *Escherichia coli* and *Proteus vulgaris*. The ligands had no activity, whereas some of the complexes were found to have antibacterial activity on some bacteria in vitro, The effective concentration of the complexes ranging between 0.01 - 10 μg/ml.

الخلاصة

درست الفعالية البايولوجية لليكندين (بنزالديهايـد ثايوسـميكاربازون-BTSCH و4،3 - ثنـائي ميثوكسي بنزالدازاين-DMBA) ولمعقداتهما مع الخارصين(II) والكادميوم(II) والزئبق(II) ذات الـصيغ العامة $M_2(BTSCH)_2(DMBA)_2X_4$ و M(BTSC)(DMBA)X (حيث BTSC=الليكنـد BTSCH مزال منه بروتون ، Zn^{2+} = M او Cd^{2+} او Hg^{2+}، X = NO_3^- او Cl^- او I^-) في محلـول ثنـائي ميثيل سلفوكسيد (DMSO)على خمسة من الجراثيم البشرية المرضية باستخدام تقنية الانتشار علـى سـطح الاكار كمضادات للبكتريـا *Staphylococcus aureus* و *Bacillus subtilis* و *Klebsella pneumonia* و *Escherichia coli* و *Proteus vulgaris*. وقد اثبتت الدراسة في الوسط الصنعي ان الليكندات ليـست لها فعالية، في حين ان بعضا من المعقدات قيد الدرس لها فعالية كمضادات لبعض الجراثيم في حين بعـضها الاخر فلا يكون فعالا. يتراوح التركيز المؤثر بين 0.01 – 10 مايكرو غرام / مل.

Part I: Some new complexes of Zn(II), Cd(II) and Hg(II) containing mixed ligands. Accepted in first science conference, Mosul University, College of Science, Chemistry Department, 2008

Introduction

Zinc is one of the most common elements in the earth's crust. It's found in air, soil, water and all foods, breathing large amount of zinc (as dust or fumes) can cause a specific short–term disease called metal fume fever. Exposure to zinc compound can cause skin irritation. Eating large amounts of zinc, even for a short time, can cause stomach cramps, nausea and vomiting [(1)].

The administration of zinc and cadmium to animals induced the synthesis of metallothioneins proteins, which play an important role in the metabolism of these elements[(2).] zinc is the most common trace element and the only known metal required for at least one enzyme in each of the major classes of enzymatic activities, and sited in enzymes into structural and catalytical sites. Zinc play multifaceted role in biological system[(3)]. Zinc(II) complexes with Schiff base ligands[(4)] and thiosemicarbazone ligands[(5)] had antimicrobially active against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Cadmium is known carcinogen that inactivate the DNA mismatch repair pathway. It is highly inhibitory to ATP binding and less inhibitory to its DNA mismatch binding activity[(6).] Cadmium(II) complexes had antimicrobial activity against pathogenic fungus *Candida albicans* and pathogenic gram-negative *(Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus, Enterococcus faccalis*)[(7)].

Mercury is an extremely toxic element, it combines chemically with enzyme in the body functions. A compound of mercury is once used in the manufacture of fell hats, workers with this compound gradually began to develop serious problems, including the loss of hair and teeth, loss of memory and a general deterioration of the nervous system. This leads to the common expression made as hatter for bizalle behaviour[(8)]. The very properties that made mercury poisonous to humans made it effective in dealing with insect pests. $HgCl_2$ is poison used as fungicide and pesticide. HgCl is used in agriculture to control root maggots and the pests on tubers and bulbs. Although longer used in medicine, it was used once as a purgative treatment syphilis[(8)]. Mercury (II) complexes with 2-formylpyridinethiosemicarbazone showed good anti-amoebic activity[(9)] and their complexes with isatin-3-thiosemicarbazone showed good and antimicrobial activity[(10)].

Thiosemicarbazones comprised a well–known group of nitrogen and sulfur donors, they aroused considerable interest due to their pharmacological properties, antibacterial, antitumor and antifungal activities [(11-16)].

Azine represent a well know class of organic compounds used in different biological applications [(17-19)].

Mixed ligand complexes have been receiving considerable attention largely due to their considerable importance in the field of the metalloenzymes and other biological activities [(20-24)].

Due to the importance of mixed ligand complexes we took a humble part in the coordination chemistry of mixed ligands and their biological activities, and some articles were published [(25-27)].

In view of this and since the biological activities mixed ligands–zinc(II) or cadmium(II) or mercury(II) complexes have not been reported yet, it is a matter of interest to determine the extent of these complexes on the bacterial growth.

In the present work, the antibacterial activity of the ligands and their cadmium(II), zinc(II) and mercury(II) complexes have been studied against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsella pneumonia*, *Escherichia coli* and *Proteus vulgaris*.

Experimental

1- Materials and apparatus:

All the chemicals have been used as supplied (Fluka or BDH or Aldrich) except benzaldehyde which was used after purification by distillation.

The melting or decomposition points were measured by Richert-Jung Heizubank type WME. Elemental analysis were carried out by precipitation methods. Molecular weights have been determined cryoscopically. Molar conductivities have been measured using Electrolytic conductivity measuring set type LF-42. Infrared spectra were measured by FT-IR Bruker type Tensor27. Electronic spectra have been carried out by Shimadzu UV-1650 PC UV-Visible Spectrophotometer.

2-Preparation of the ligands :

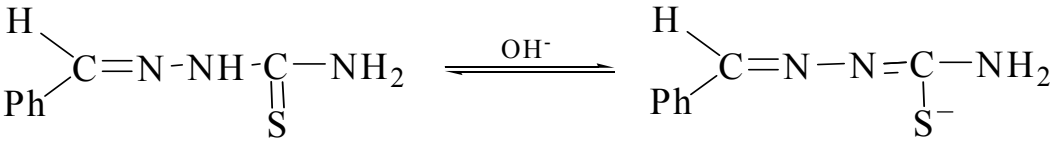
Thiosemicarbazone-BTSCH ligand (Figure 1) has been prepared according to previous method [28,29], it has been prepared by refluxing equimolar quantities of the thiosemicarbazide, benzaldehyde and sodium acetate solution for about 3 hours. The thiosemicarbazone thus formed has been filtered off from it's solution, Washed with distilled water and recrystallized from ethanol (m. p.= 330^{o} c).

Azine ligands (figure 1) has been prepared according to previous method [28, 30]. A mixture of hydrazine sulphate in 18 ml water and 2.4 ml concentrated NH_4OH solution have been stirred. The corresponding amount of 3,4-dimethoxybenzaldehyde has been added with continueous stirring over a period 30 – 60 minutes. The mixture has been stirred for a further hour. The solid product was filtered off, washed with water, recrystallized from ethanol. Yellow crystals were obtained (m. p.=174 ^{o} c).

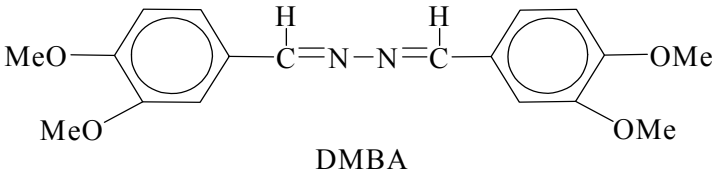
3-Preparation of the complexes :

Complexes (figure 1) of the type $M_2(BTSCH)_2(DMBA)X_4$ (where BTSCH= benzaldehydethiosemicarbazone; DMBA= 3,4-dimethoxybenzaldazine, X = NO_3^- or Cl^- or I^-, M = Zn^{2+}, Cd^{2+}, or Hg^{2+}) have been prepared[28] by the reaction of MX_2 with ethanolic solution of benzaldehydethiosemicarbazone and 3,4-dimethoxybenzaldazine in 2:2:2 molar ratio. The mixture has been refluxed for 3 hours, evaporated to about half their volumes and then cooled. The resulting product has been filtered off, washed with diethylether and dried.

Complexes (figure 1) of the type Zn(BTSC)(DMBA)X have been prepared [28] by the reaction of aqueous solution of metal salts with ethanolic solution of benzaldehydethiosemicarbazone and 3,4-dimethoxybenzaldazine in 1:1:1 molar ratio. Sodium hydroxide solution (1 M) has been added to the mixture until PH ≈ 8 – 9. The resulted products were filtered off, washed with diethylether and dried.



BTSCH BTSC



DMBA

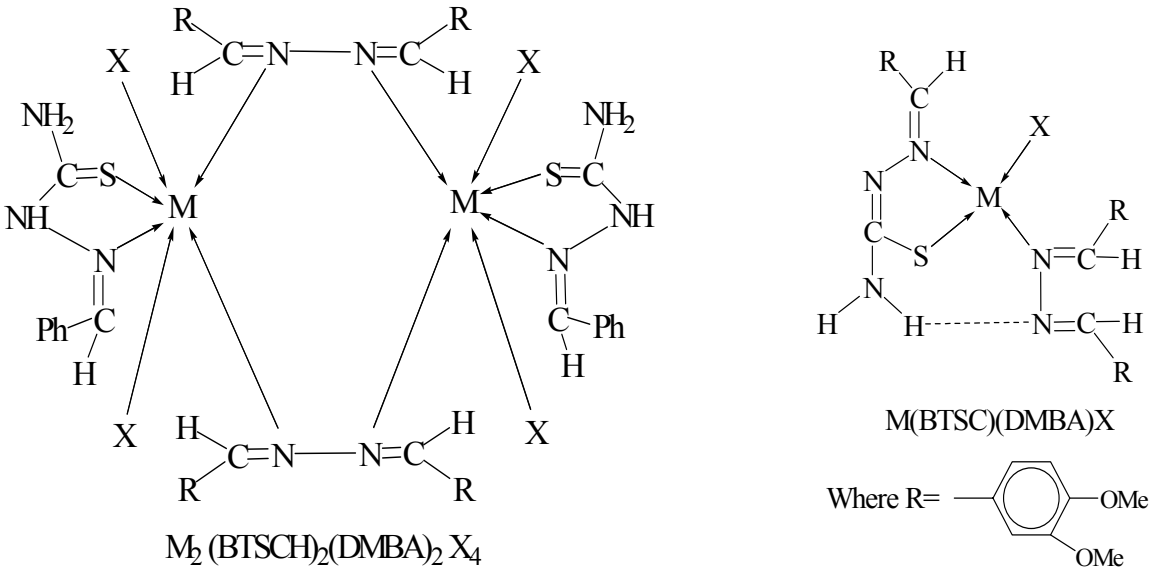


Figure 1 : Structures of the ligands and their complexes

4- Antimicrobial assay of the complexes :

Five pathogenic microorganisms bacteria have been selected to study the antibacterial activity of the ligands and their complexes in this research. These were *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsella pneumonia*, *Escherichia coli* and *Proteus vulgaris*. All the bacterial strains have been obtained from University of Mosul, College of Education, Biology Department. The antibacterial activity has been evaluated by applying Leven et al.[31]method that depended on Vandepitte et al.[32]. Nutrient agar was incubated by using single colony of the five types of bacteria a foresaid singly, then the media was incubated at 37°c for 24 hrs[33-35]. The microbial suspension was diluted by normal saline solution by comparison with standard test tube (Macferland No. 1). It contained 10^8 cell/cm^3 from the microbial suspension. Then it was spread on agar media surface by using glass spreader, the dishes were incubated for 30 minutes until the absorption has been completed. Then, the dishes were prepared from filter paper (Whatman No. 1) diameter (6mm) and saturated by different concentrations of the different ligands and complexes solutions (10, 1, 0.1, 0.01 µg/ml of each ligand or complex solution) and then dried, dry dimethylsulphoxide has been used for the antibiotics (Chloramphinicol and Erethromycin). Blank paper discs of dimethylsulphoxide have been used as control. The inoculated plate have been incubated at 37°c for 24 hours and inhibition zones were measured[36] in all experiments the mean of each triplicate was measured[37]. The zone of inhibition have been measured for the determination of the minimum inhibitory concentration (MIC) the highest dilution which inhibits the growth were recoverded (figure 2).

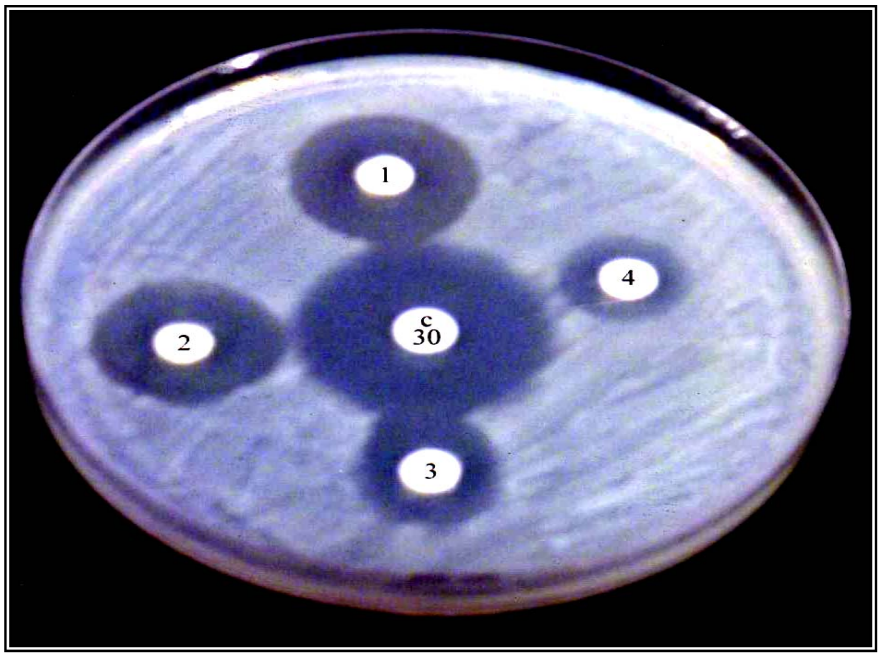


Figure 2 : Antimicrobial activity of different concentrations of complex 12 on *Klebsella pneumonia*

Results and Discussion

Many chemical compounds had good abilities to attack the bacteria through their effects on the synthesis of ribonucleic acid which could be resulted from the inhibition action of these compounds on the DNA of the bacteria which caused the inhibition of the activities of DNA gyrase enzyme including the separation of supercoiling or decantenation or unkotting of DNA[38-41]. Moreover, the antibacterial agent were known to attack the cell in a variety of ways such as killing or inhibiting the growth of microorganisms by affecting special target sites like the synthesis of cell wall, protein and nucleic acid or by inhibiting the function of the cell membrance, bindings of the sulfhydryl groups of the cell enzymes with the complexes[19]. Numerous experiments have been done to determine the antimicrobial influence of the complexes Table 1 showed that the ligands BTSCH and DMBA have no activity against all the bacteria under investigation. The complexes 7, 8, 9, 11, 12 and 13 have antimicrobial activity against all the microorganisms under investigation. Complex 10 has moderate activity against all the microorganisms, where as complexes 3 and 6 have moderate antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. As metal ion perferentially bind to SH group of the cell enzyme more strongly, it is logical to assume that the complexes screened were involved in competitive equilibria involving the SH group of the cell enzyme. Therefore, we concluded that most of the complexes aquire biological activity. If this is the case, the complexes which were expected to bind to SH group of the cell enzyme acted more strongly than the nitrogen donor atom[42] in the ligands (Table 1), these observation have been consistent with that observed by many workers[43]. The concentrations of the complexes ranging between 0.01–10 μg/ml. The effective concentration of some complexes

showed greater activity than the control, other showed same activity as the control, whereas some of the complexes under investigation have no activity (Table 2).

Conclusion :

From this study we could conclude the following:

1- The ligands BTSCH and DMBA showed no antibacterial activity.

2- Complexes 7, 8, 9, 11, 12 and 13 showed some times greater activity than the control and sometimes showed same activity as the control.

3- Complexes 4 and 6 showed less activity than control against only *Staphylococcus aureus* and *Bacillus subtilis*, whereas no activity was observed on the other microorganisms.

4- Complex 10 showed less activity against all the bacteria under investigation.

5- The other complexes 1, 2, 3 and 5 did not show any activity against all the bacteria.

Table 1 : Antibacterial activity of the complexes

No	Complexes	*Staph. aureus*	*Bac. subtilis*	*K. Pneumonia*	*Esch. coli*	*Prot. vulgaris*
	BTSCH	R	R	R	R	R
	DMBA	R	R	R	R	R
1	$Zn_2(BTSCH)_2(DMBA)_2I_4$	R	R	R	R	R
2	Zn(BTSC)(DMBA)X	R	R	R	R	R
3	$Zn_2(BTSCH)_2(DMBA)Cl_4$	R	R	R	R	R
4	Zn(BTSC) (DMBA)Cl	MS	MS	R	R	R
5	$Zn_2(BTSCH)_2(DMBA)_2(NO_3)_4$	R	R	R	R	R
6	Zn(BTSC)(DMBA) NO_3	MS	MS	R	R	R
7	Cd (BTSC) (DMBA) I	S	S	S	S	S
8	Cd_2 $(BTSCH)_2$ $(DMBA)_2$ Cl_4	S	S	S	S	S
9	Cd (BTSC) (DMBA) Cl	S	S	S	S	S
10	$Cd_2(BTSCH)_2(DMBA)_2(NO_3)_4$	MS	MS	MS	MS	MS
11	Cd(BTSC) (DMBA) NO_3	S	S	S	S	S
12	Hg (BTSC) (DMBA) I	S	S	S	S	S
13	$Hg_2(BTSCH)_2(DMBA)_2(NO_3)_4$	S	S	S	S	S
Control	Chloramphinicol (30 μmg)	S	S	S	S	S
	Erethromycin (15 μg)	S	S	R	S	S

S = Sensitive diameter not more than 6 mm less than control [36, 44]. MS = Moderate sensitive zone diameter of 6 – 12 mm less than control. R = Resistant zone diameter of 12 mm or less than control.

Table 2 : Antibacterial activity (inhibition zone 6 mm) of different concentration of the complexes (µg / ml)

No	*Staph. aureus*				*Bac. subtilis*				*K. Pneumonia*				*Esch. coli*				*Prot. vulgaris*			
	10	**1**	**0.1**	**0.01**	**10**	**1**	**0.1**	**0.01**	**10**	**1**	**0.1**	**0.01**	**10**	**1**	**0.1**	**0.01**	**10**	**1**	**0.1**	**0.01**
BTSCH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DMBA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	10	-	-	-	12	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	10	-	-	-	13	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	19	14	8	-	22	15	11	7	21	15	10	7	24	19	13	8	22	15	10	7
8	20	16	8	-	21	16	11	7	23	17	10	7	22	16	10	7	21	15	10	7
9	22	18	10	8	23	19	12	7	23	18	4	7	21	15	10	7	22	17	10	7
10	11	8	-	-	12	8	-	-	14	8	-	-	13	8	-	-	12	8	-	-
11	25	20	12	8	22	16	11	7	28	19	12	8	21	16	11	7	23	18	12	7
12	26	20	16	12	22	16	12	7	23	17	12	8	19	13	7	-	24	19	13	7
13	22	16	11	8	19	13	8	-	23	18	13	8	18	12	8	-	24	19	13	8
Chl.	21				20				25				22				23			
Ereth	22				23				-				18				17			

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*The complexes under investigation have previously characterized by elemental analysis, molecular weight determination, molar conductance values spectral data (infrared and electronic spectra) [28].