BIOLOGICAL ACTIVITY OF SOME MIXED LIGAND COMPLEXES – PART II[+]

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Abstract

The biological activity of some zinc (II) complexes containing mixed ligands having the formulaes [Zn(BBSCH)(LH)X] and [Zn(BBSC)(L)] (where BBSCH = benzilbis- (semicarbazone), $BBSC^-$ = deprotonated BBSCH ligand, LH = the ligands benzoinsemi- carbazone-BSH or 2-chlorobenzaldehydesemicarbazone-CSH or 2-fluorobenzaldehyde- semicarbazone-FSH or furaldehydesemicarbazone-FrSH, L = deprotonated LH ligand, X = SO_4^{2-} or CO_3^{2-}) in dimethylsulphoxide solution have been evaluated by agar plate diffution technique against five human pathogenic bacterial strains = *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*. The complexes were found to have antibacterial activity on some bacteria in vitro. The effective concentration of the complexes ranging between 1-10 µg/ml. Bacillus subtilis **and *Proteus mirabilis* were the most sensitive bacteria followed by *Staphylococcus aureus*.**

المستخلص

تمت دراسة الفعالية لبعض معقدات الخارصين (II) الحاوية على مزيج من الليكندات ذات الصيغ العامة [Zn(BBSCH)(LH)X] و [Zn(BBSC)(L)] (حيث BBSCH = بنزل بس(سميكاربازون)، $BBSC^-$ = الليكند BBSCH مزال منه بروتون، LH = الليكندات بنزوين سميكاربازون–BSH او ٢–كلوروبنزالديهايد سميكاربازون–CSH او ٢–فلوروبنزالديهايد سميكاربازون– FSH او فورفورالديهايد سميكاربازون–FrSH ، L = الليكند LH مزال منه بروتون، X = SO_4^{2-} او CO_3^{2-}) في محلول ثنائي ميثيل اوكسيد الكبريت على خمسة من الجراثيم البشرية المرضية باستخدام تقنية الانتشار على سطح الاكار كمضادات للبكتريا *Bacillus subtilis* و *Staphylococcus aureus* و *Pseudomonas aeruginosa* و *Escherichia coli* و *Proteus mirabilis* ، وقد اثبتت الدراسة في الوسط الصنعي ان هذه المعقدات لها فعالية كمضادات لبعض الجراثيم، يتراوح التركيز المؤثرللمعقدات بين ١–١٠ مايكروغرام /مل. ان البكتريا *Bacillus subtilis* و *Proteus mirabilis* هما الاكثر حساسية للمركبات وتلتها بكتريا *Staphylococcus aureas* .

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Introduction

The coordination chemistry of zinc in both non-biological and biological areas has been the subject of intensive researches. The administration of zinc to animals induced the synthesis of the proteins called metallothioneins, which played an important role in the metabolism of this element[1]. Zinc is the most common trace element and the only metal known to be required for at least one enzyme in each of the major classes of enzymatic activities. Zinc plays multifaceted roles in biological system[2].

Some aromatic compounds possessed antimicrobial activities against some bacteria [3]. Schiff bases have been shown to possess antibacterial activity [4,5] and their complexes demonstrated good or remarkable activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* [6]. Schiff bases and their complexes have received renewed attention recently, due to their antitumor, antimicrobial and antifungal activities. They were known to exhibit a wide variety of pharmacological properties (e.g. antiflammatory, antimalarial, antihelmintic, anticonvulsant activity and hypotensive action) [7,8].

Substituted thiadiazole, substituted oxadiazole possessed various biological activities such as antitumor, antibacterial and antimicrobial activity [9]. Thiosemicarbazone complexes possessed antitumor activity in addition to other activities [10-12]. Semicarbazones and their complexes had important biological applications [13,14]. On the other hand mixed ligand complexes had considerable importance in the field of metalloenzymes and other biological activities [15-17].

Due to the importance of mixed ligand complexes, we took a humble part in the chemistry of mixed ligands and their biological activity, and some articles have been published [18,19].

In view of this and since the biological activities of mixed ligand-zinc (II) complexes have not been reported yet, it is a matter of interest to determine the extent of these complexes on the bacterial growth. The complexes under investigation have been previously characterized by elemental analysis, molecular weight determination, molar conductance values, magnetic moment data, infrared spectrophotometry and electronic spectral data [20].

In the present work, the antibacterial activity of some zinc (II)-mixed ligand complexes have been studied against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*.

Materials and Methods

All the chemicals have been used as supplied from Fluka or BDH. Augmentin and Ciprofloxacin have been used as supplied from Biomerieux France.

1. Synthetic methods

Semicarbazones ligands (Figure 1) have been prepared according to the literature method [20,21]. Benzilbis(semicarbazone) has been prepared [21] by refluxing 1:2 molar ratio of benzil : semicarbazide hydrochloride and sodium acetate

solution for about 3 hours. The semicarbazone thus formed has been filtered off from it's solution, washed with distilled water and recrystallized from ethanol.

The ligands BSH, CSH, FSH and FrSH (Figure 1) have been prepared [20] by refluxing equimolar quantities of the semicarbazide hydrochloride and the corresponding aldehyde. Same procedure has been followed as above.

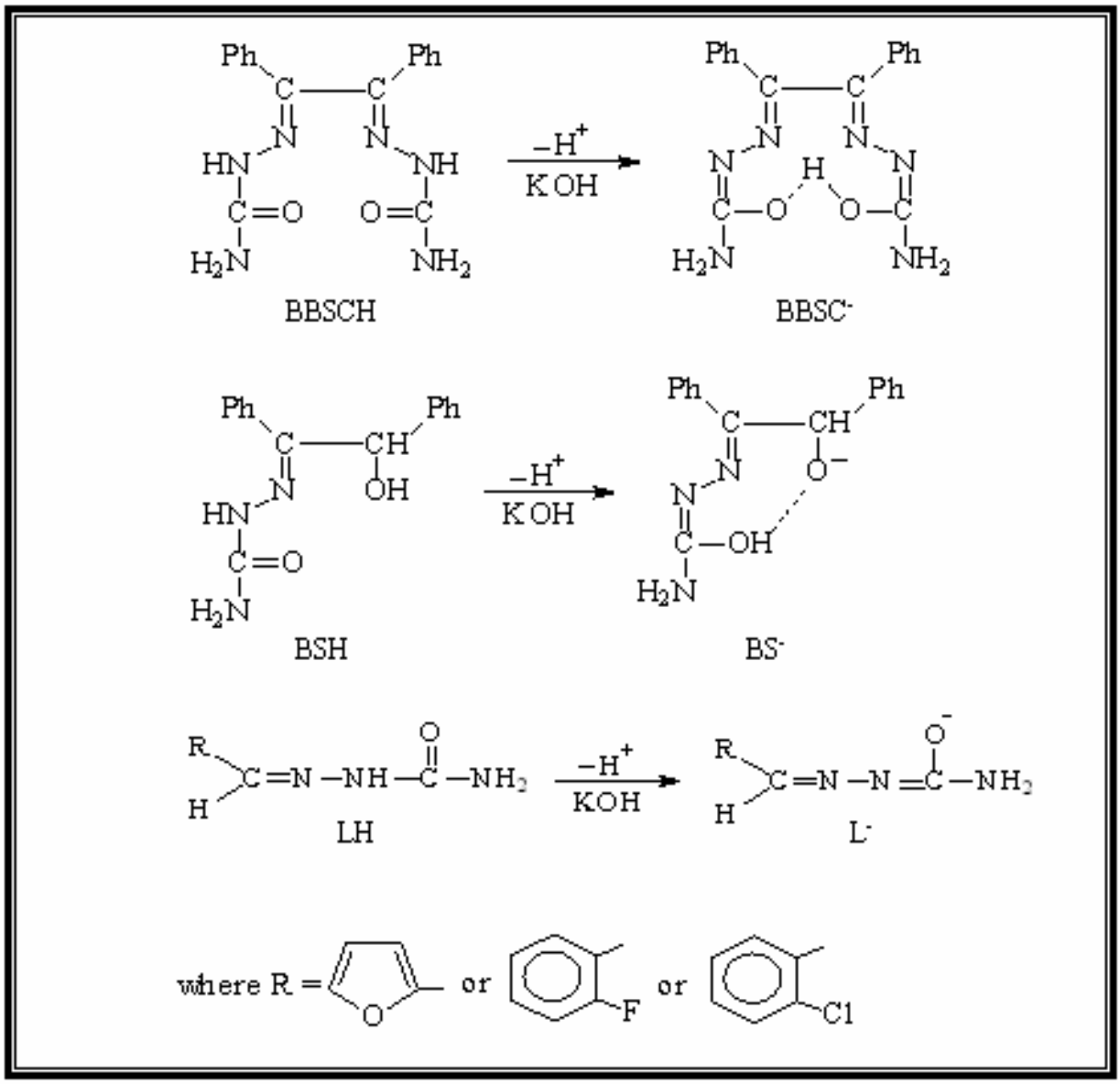


Figure 1: Structures of the ligands

2. Preparation of zinc (II) complexes [20]

Complexes (Figure 2) of the type [Zn(BBSCH)(LH)X] (where LH = BSH, FSH, FrSH or CSH, X = SO_4^{2-} or CO_3^{2-}) have been prepared by the reaction of 0.0018 mole $ZnSO_4.7H_2O$ or 0.0040 mole $ZnCO_3$ with 50 ml ethanolic solution of benzilbis(semicarbazone) and the other semicarbazone ligands in 1:1:1 molar ratio. The mixture has been refluxed for 3 hours, evaporated to about half it's volume and cooled. The resulting product has been filtered off, washed with petroleum ether and dried.

Complexes (Figure 2) of the type [Zn(BBSC)(L)] (where L = deprotonated LH ligands, $BBSC^-$ = deprotonated BBSCH ligand) have been prepared by the reaction of 0.0018 mole $ZnSO_4.7H_2O$ or 0.0040 mole $ZnCO_3$ with benzilbis(semicarbazone) and other semicarbazone ligand in 1:1:1 molar ratio. Ethanolic solution of sodium hydroxide (1M) has been added to the mixture until pH = 8-9. The products have been filtered off, washed with petroleum ether and dried.

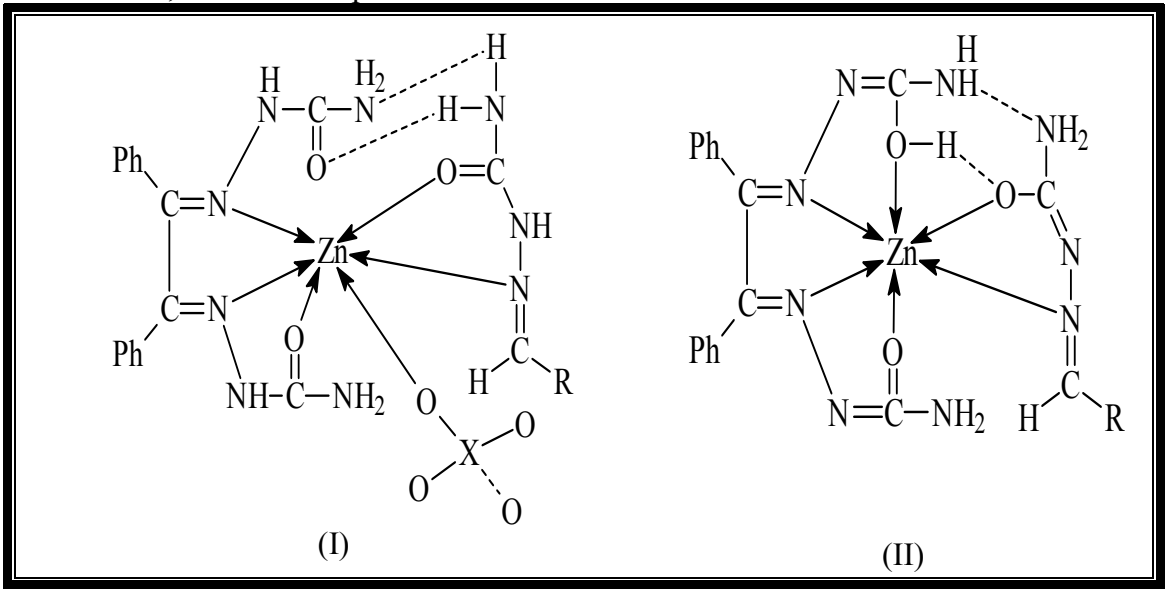
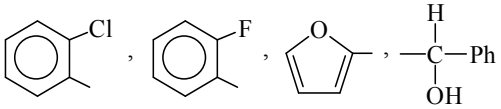


Figure 2: Structures of the complexes
(I) complexes in neutral medium. (II) complexes in basic medium
X = S or C, ... O = O or nothing

R = 

3. Antimicrobial assay of the complexes

Five pathogenic bacteria have been selected to study the antibacterial activity of the complexes in this research. These were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*. All the bacterial strains have been isolated and identified using standard method [22] before use in Department of Biology, College of Education, University of Mosul. They grown up to the stationary phase in nutrient broth at 37 ^oC and a sample of 0.5 ml of each bacteria was spread over the surface of nutrient agar plate [23].

Discs of filter paper (6 mm diameter) have been sterilized at 140 ^oC for 1 hour and impregnated with 1 ml containing the concentrations 10, 1.0, 0.1 and 0.01 μg/ml of each complex solution and then dried, dry dimethylsulphoxide has been used for the antibiotics (Augmentin and Ciprofloxacin). Blank paper discs of dimethylsulphoxide have been used as control. The inoculated plate have been incubated at 37 ^oC for 24 hours and inhibition zones were measured [24] in all experiments the mean of each triplicate was measured [25].

Results and Discussion

Many chemical compounds had a good ability 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 inhibition of the activities of DNA gyrase enzyme including the separation of

supercoiling or decantenation or unkotting of the DNA [26-29]. 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 membrane, binding of the sulfhydryl groups of the cell enzymes with the complexes [18]. Numerous experiments have been done to determine the antimicrobial influence of the complexes. Table 1 showed that the complexes 1,8,9 and 12 have antimicrobial activity against only *Bacillus subtilis*, whereas complexes 2-7 and 15 have antimicrobial activity against *Bacillus subtilis* and *Proteus mirabilis*, while complexes 10, 11, 14 and 16 have antimicrobial activity against both *Staphylococcus aureus* and *Proteus mirabilis*. Meanwhile complex 13 has antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus mirabilis*. As metal ion preferentially 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 in the ligand (Table 1) [30], these observation have been consistent with that observed by many workers [11].

Table 1: Antibacterial activity of the complexes

No.	Complexes	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus mirabilis*
1	$[Zn(BBSCH)(BSH)CO_3]$	MS	R	R	R	R
2	[Zn(BBSC)(BS)]	MS	R	R	R	S
3	$[Zn(BBSCH)(CSH)CO_3]$	MS	R	R	R	S
4	[Zn(BBSC)(CS)]	S	R	R	R	S
5	$[Zn(BBSCH)(FSH)CO_3]$	S	R	R	R	S
6	[Zn(BBSC)(FS)]	MS	R	R	R	S
7	$[Zn(BBSCH)(FrSH)CO_3]$	S	R	R	R	MS
8	[Zn(BBSC)(FrS)]	S	R	R	R	R
9	$[Zn(BBSCH)(BSH)SO_4]$	MS	R	R	R	R
10	[Zn(BBSC)(BS)]	R	MS	R	R	S
11	$[Zn(BBSCH)(CSH)SO_4]$	R	MS	R	R	MS
12	[Zn(BBSC)(CS)]	MS	R	R	R	R
13	$[Zn(BBSCH)(FSH)SO_4]$	MS	MS	R	R	MS
14	[Zn(BBSC)(FS)]	R	MS	R	R	MS
15	$[Zn(BBSCH)(FrSH)SO_4]$	MS	R	R	R	MS
16	[Zn(BBSC)(FrS)]	R	MS	R	R	S
control	Augmentin (30 μg)	MS	S	R	R	R
	Ciprofloxacin (5 μg)	R	R	S	S	S

S = sensitive diameter not more than 6 mm less than control [31,31]
M = moderate sensitive zone diameter of 6-12 mm less than control
R = resistant zone diameter of 12 mm or less than control

The concentration of the complexes ranging between 1-10 μg/ml. The effective concentration of complexes 4, 5 and 7 (10 μg/ml) showed greater activity against *Bacillus subtilis* than the control (Augmentin), whereas the effective concentration of complex 8 (10 and 1.0 μg/ml) showed greater activity against *Bacillus subtilis* than the control (Augmentin), The effective concentration of complexes 2, 3 4, 5, 6, 10 and 16 (10 μg/ml) showed greater activity against *Proteus*

mirabilis than the control (Ciprofloxacin), whereas complexes 2, 4 and 6 ((1.0 μg/ml) showed same activity against *Proteus mirabilis* as the control (Ciprofloxacin)

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

No.	***Bacillus subtilis***				***Staphylococcus aureus***				***Pseudomonas aeruginosa***				***Escherichia coli***				***Proteus mirabilis***			
	Inhibition zone/6mm				**Inhibition zone/6mm**				**Inhibition zone/6mm**				**Inhibition zone/6mm**				**Inhibition zone/6mm**			
	10	**1.0**	**0.1**	**0.01**	**10**	**1.0**	**0.1**	**0.01**	**10**	**1.0**	**0.1**	**0.01**	**10**	**1.0**	**0.1**	**0.01**	**10**	**1.0**	**0.1**	**0.01**
1	15	10	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	15	9	1	1	-	-	-	-	-	-	-	-	-	-	-	-	20	18	16	13
3	15	8	1	1	-	-	-	-	-	-	-	-	-	-	-	-	20	16	13	10
4	20	18	15	9	-	-	-	-	-	-	-	-	-	-	-	-	22	18	11	9
5	22	18	15	11	-	-	-	-	-	-	-	-	-	-	-	-	20	13	10	1
6	14	9	1	1	-	-	-	-	-	-	-	-	-	-	-	-	22	18	15	13
7	20	18	15	11	-	-	-	-	-	-	-	-	-	-	-	-	15	10	9	1
8	23	20	15	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	10	8	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	15	12	9	1	-	-	-	-	-	-	-	-	20	17	15	13
11	-	-	-	-	12	8	1	1	-	-	-	-	-	-	-	-	12	8	1	1
12	15	12	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	15	11	9	1	15	13	10	1	-	-	-	-	-	-	-	-	15	13	9	1
14	-	-	-	-	16	10	1	1	-	-	-	-	-	-	-	-	10	1	1	1
15	15	13	9	1	-	-	-	-	-	-	-	-	-	-	-	-	10	1	1	1
16	-	-	-	-	15	13	12	9	-	-	-	-	-	-	-	-	20	13	9	1
Aug.	19				20				-				-				-			
Cip.	-				-				16				18				18			

Conclusion

From the above data we could conclude the following:

1. Complexes 1,2,3,6,9,12,13 and 15 showed less activity against *Bacillus subtilis* than the control (Agumentin), whereas complexes 10,11,14 and 16 showed no activity against the same bacteria.
2. Complexes 10,11,13,14 and 16 showed less activity against *Staphylococcus aureus* than the control (Augmentin), whereas all the other complexes have no activity against the same bacteria.
3. All the complexes did not show any activity against *Pseudomonas aeruginosa* and *Escherichia coli*.
4. Complexes 1,8,9 and 12 did not show any activity against *Proteus mirabilis*, whereas complexes 11,13,14 and 15 showed less activity than the control (Augmentin) against the same bacteria.
5. Complexes 4,5,7 and 8 showed a good activity against *Bacillus subtilis*, i.e greater activity than the control (Augmentin).
6. Complexes 2-6,10 and 16 showed greater activity against *Proteus mirabilis* than the control (Ciprofloxacin).

References

1. Wilkinson G., Gillard R.D. and McCleverty J.A., Comprehensive Coordination Chemistry, 4th ed., Pergamon Press, Great Britain, V5, pp. 926, 1021, 1987.

2. Penner-Haha J.E., Kelly R.A., Tobin D.A., Weng T.C. and Fierk C.A., "Zinc containing alkyl-transfer enzymes a new class of biological Zn site", 87th Canadian Chem. Conferences and Exhibition, Hill-Gballroon W., Tartarus, pp. 358, 2004.
3. Ahmad A., Khan K.A., Sultana S., "Study of the in vitro antimicrobial activity of harmin, harmaline and their derivatives", J. Ethnopharmacology, Vol. 35, pp. 289, 1992.
4. Hameed A.S. and Saleh, "Studies on 1,3,4-thiadiazoles. Part II. Synthesis, antimicro- bial activity and liquid crystal properties of some new Schiff base derived from (4,4`-alkoxy benzoyloxy)benzaldehyde", National J. Chem., Vol. 5, pp. 121, 2000.
5. Ismail K.Z., "Synthesis, spectroscopic, magnetic and biological activity studies of copper (II) complexes of an antipyrine Schiff base", Trans. Metal Chem., Vol. 25, No. 5, pp. 522, 2000.
6. Shrivastava A., Singh N.K. and Singh S.M., "Synthesis, characterization and antitumor studies of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of N-salicyloyl-N-o-hydroxy thiobenzhydrazide", Bioorg. Med. Chem., Vol. 10, No. 4, pp. 887-895, 2002.
7. Yan S., Burton W.H., Chien P.L. and Cheny G.C., "Potential causal antimallerial agents", J. Heterocyclic Chem., Vol. 15, pp. 247, 1976.
8. Aminabbayi T.M., Biradar N.S., Patil S.P. and Hoffman D.E., "Biological studies on benzimidazolyl amino acid complex of dimethyldichlorosilane", Inorg. Chem. Acta, Vol. 125, pp. 125, 1986.
9. Daoud K.M., Sulyman Kh.D. and Muhammad M.J., "Synthesis and biological study of some hydrazides, thiosemicarbazides and 1,3,4-thiadiazole", Tikrit J. Pure Sci., Vol. 10, No. 1, pp. 157-161, 2005.
10. Afrasiabi Z., Sinn E. and Padhye Sh., "Transition metal complexes of phenathene- quinone thiosemicarbazone as potential anticancer agents", J. Inorg. Biochem., Vol. 95, pp. 306, 2003.
11. Nadira V. and Singh H.B., "Vitro evaluation of bacteriostatic activity of metal complexes", Inorg. Chim. Acta, Vol. 151, pp. 387, 1988.
12. Ferrari M.B., Bisceglie F., Pelosi G., Tarasconi P., Albertini R. and Botani A., "Synthesis, characterization, X-ray structure and biological activity of the new 5-formyl uracil thiosemicarbazone complexes", Inorg. Biochem., Vol. 83, pp. 169, 2001.
13. Patole J., Padhye S., Newton C.J., Anson C.E. and Powell A.K., "Synthesis, characterization and in vitro anticancer activities of semicarbazone and thiosemicarbazone derivatives of salicylaldehyde and their copper complexes against human breast cancer cell line MCF-7", Inorg. Chim. Acta, Vol. 318, pp. 1654-1658, 2004.
14. Afrasiabi Z., Sinn E., Kulkarni P.P., Ambike V., Padhye S., Deobagakar D., Anson C.E. and Powell A.K., "Synthesis and characterization of copper (II) of 4-alkyl/aryl-1,2-naphthoquinones thiosemicarbazone derivatives as potent DNA cleaving agents", Chem. Acta, Vol. 358, pp. 2023-2030, 2005.
15. Reddy K.H., Reddy P.S. and Babu P.R., "Nuclease activity of mixed ligand complexes of copper (II) with heteroaromatic derivatives and picoline", Trans. Metal Chem., Vol. 25, pp. 505-510, 2000.
16. Bindu P., Kurup M.R.P. and Satyakeerty T.R., "EPR, cyclic voltametric and biological studies of copper (II) complexes of salicylaldehyde N(4) substituted

thiosemicarbazone and heterocyclic bases", Polyhedron, Vol. 18, pp. 321, 1998.
17. Ramesh R. and Sivamasundari M., "Synthesis, spectral and antifungal activity of mixed ligand complexes", Synth. React. Inorg. Met-Org. Chem., Vol. 33, No. 5, pp. 899-910, 2003.
18. Dawood Z.F., Shareef A.Y. and Al-Shama'a M.A., "Biological activity of some nickel (II) complexes", Sci. Kerbala Univ. J., Accepted in 2006.
19. Dawood Z.F., Shareef A.Y. and Ahmad N.G., "Biological activity of Ru(III) and Pd(II) complexes", Under Press.
20. Dawood Z.F. and Jasim Z.U., "Preparation of some new Zn(II) complexes containing mixed ligands of semicarbazones", Tikrit J. Pure Sci., Accepted in 2006.
21. Vogel A.I., "Textbook of Practical Organic Chemistry", 3rd ed., Longman Green, London, pp. 344, 1964.
22. Colins C.H., Lyne P.M. and Jrange J.M., "Microbiological Method", 6th ed., Buttarworth, pp. 59, 1989.
23. Konemon E.W., Allen S.D., Janda W.M., Scheekenberg P.C. and Winn W.C., "Color Atlas & Text Book of Diagnostic Microbiology""5th ed., J.B. Lippincott Com., Philadelphia, New York, 1997.
24. Garrod L.P., Lambert H.P., Grady D. and Water-Worth P., "Antibiotic and Chemotherapy", 5th ed., Churchill Livingston, N.Y., 1981.
25. Seely H.W. and Vandemerk Jr.P.J., "Microles in Action", 3rd ed., Freeman and Company, 1981.
26. Katzung B.G., "Chemotherapeutic Drugs. In Basic and Clinical Pharmacology", 4th ed., Hall International Inc., pp. 545-551, 1989.
27. Piddock L.J.V., Walters R.N. and Driver J.M., "Correlation of quinolone MIC and inhibition of DNA, RNA and protein synthesis and induction of SOS response in Escherichia coli", Antimicrob. Agents and Chemother., Vol. 34, No. 12, pp. 2331-2336, 1990.
28. Wang J.C., "DNA topoisomerases: nature's solutions to the topological ramifications of the double-helix structure of DNA", Harvy Lect., Vol. 81, pp. 93-110, 1987.
29. Wolfson J.S. and Hooper D.C., "The fluoroquinolones structures mechanisms of action and resistance and spectra of activity in vitro", Antimicrob. Agent Chemother., Vol. 29, pp. 581-586, 1985.
30. Casas J.S., Castineiras A., Rodriguez A.M., Sanchez A., Sordo J., Vazquez-Lopez A., Pinelli S., Lungli P., Ciancianaini P., Bonati A., Aglio P.D. and Albertini P., "Synthesis, structure, spectroscopic, properties and biological activity of mixed diorganotin(IV) complexes containing pyridine-2-carbaldehydethiosemicarbazonato and diphenyldithiophosphinato ligands", J. Inorg. Biochem., Vol. 76, No. 3-4, pp. 277-284, 1999.
31. Garrod L.P., Lambert H.P. and Grady F.O., Antibiotic and Chemotherapy, Churchill Livingstone, New York, 5th ed., pp. 459, 1981.
32. Vandepitte J., Engback K., Piot P. and Heuk C., "Basic Laboratory Procedures in Clinical Bacteriology", World Health Organisation, Geneva, pp. 84-85, 1991.