Journal of Experimental Sciences Vol. 2, Issue 5, Pages 07-12 [2011]
www.jexpsciences.com

Regular Article

Preliminary in *Vitro-* Investigation on Antimicrobial Activity of Mononuclear and Dinuclear Iron (III) Complexes

S. Sujatha *, K. Balasubramanian

Post Graduate and Research, Department of Chemistry, National College, Trichirapalli-620 011, India

ABSTRACT: In *vitro* effect of two dinuclear and mononuclear iron complexes with different ligands was examined on Gram positive and Gram negative bacterial strains. Broad spectrum antibiotic oxytetracycline was used as control. The experiment was performed by the routine agar-diffusion method of bauer et al. and the method of minimum inhibitory concentrations (MICs). It was found that mononuclear complexes expressed antibacterial effect in *vitro,* especially against Gram (+) strains. The minimum inhibitory effect of Fe (NADP) Cl_2 was more pronounced.

Key words: Antibacterial effect, Mononuclear & Dinuclear iron, Schiffs base, Oxytetracyline

Introduction

Now days, more and more strains of pathogenic microorganisms, including staphylococci, streptococci and enterobacteria, become resistant to many of available antibiotics and chemotherapeutic agents[1-4]. There are three main types of non-haem iron intradiol-cleavage enzyme catechol 1, 2-dioxygenases, protocatechuate 3, 4-dioxygenasesand chlorocatechol 1, 2 -dioxygenases. Catechol 1, 2-dioxygenases are, with one or two possible exceptions[5-6] Dimers of either identical or non-identical subunits[7] with molecular mass of 30.5±34 k Da[8-9].Catechol 1, 2-dioxygenases also perform extradiol cleavage of meta-substituted substrates such as 3-methylcatechol or 3-methoxycatechol[10-11]. Chlorocatechol1, 2-dioxygenases, by contrast, do not generally perform significant extradiol cleavage of aromatic substrates[12]. Murakami et al.] [13] that there is a subfamily of catechol 1, 2-dioxygenases in Gram positive bacteria that is clearly distinct from the two subfamilies of catechol and chlorocatechol 1, 2-dioxygenases in Gram-negative bacteria. The present work is an extension to such studies and deal with the synthesis and biological evaluation (antibacterial and antifungal activity) of Fe(III) complexes of some Schiff's base derived from different aromatic /heteroaromatic carboxyaldehydes and 4,4' substituted heteroaromatic ammines. In order to get an insight into the role, the behavior of Schiff's bases have gained a great deal of attraction. The azomethine linkage of (N=CH) is a significant feature that make Schiff's base ligands the interesting candidates for biological activities as well as co-ordination / chelation with metal ions[14-16].There are data published about the antibacterial effect of iron and different iron compounds. (Diarra et al., 1996[17] Gvozdyak et al., 1996[18] Bacchi et al.,1999[19] Donde et al., 2003[20])

Material and Methods:

The experiment were performed with two complexes of mono and di-iron derivative with ligands containing catechol derivatives and 2-hydroxy naphthaldehyde Moieties like Schiff's base **1** and**2**) ;N,N bis (naphthy2-hydroxy)1,3diamminopropane(NHDAP);N,Nbis(naphthy-hydroxy) triethylenetetramine (NHTEN). **3&4,** N,Nbis(t-butylsalicylichydroxy 4,4"diamminodiphenylmethane)(TBSHDM); N,N-bis (t-butylsalicylichydroxy) 4,4" oxydianiline – (TBSHOD).

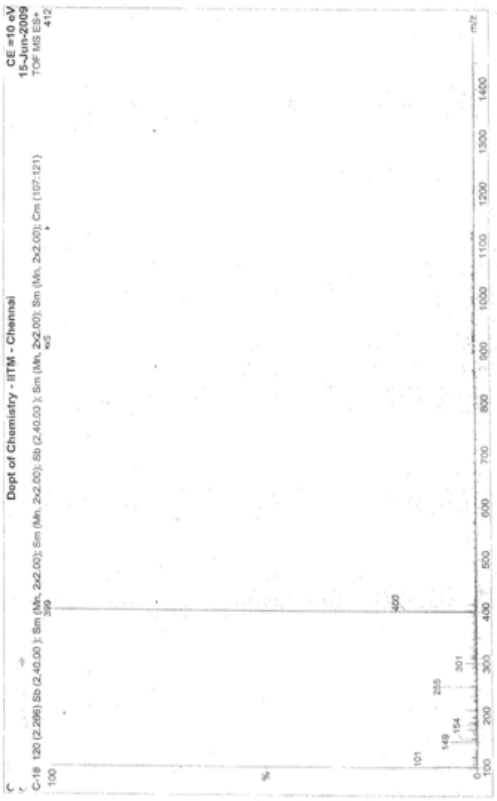
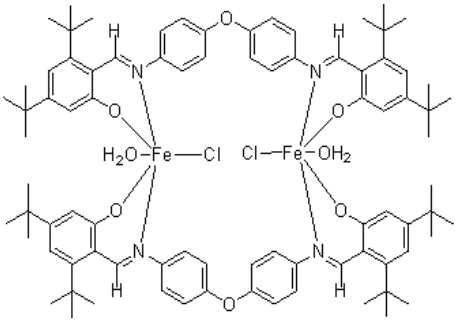


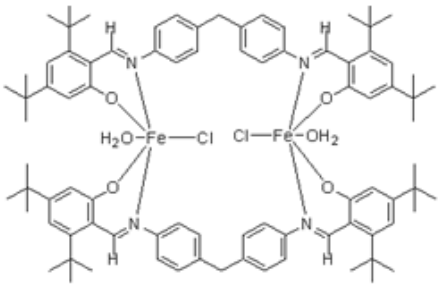
Figure 1(ligand 1 esi-ms details)

3



* Corresponding Author, Email: suja_madan@yhaoo.com

4



Experimental Section

Synthesis of ligand L1:

{N, N diphenyl methyl bis(3,5-di-tert-butylsalicylideneimine)},(L1)

3, 5di-t-butyl-2-hydroxybenzaldehyde (0.468, 2. 0mmmol) in ethanol

(100ml) was mixed with 4, 4 diamminodiphenylmethane (0.198, 1. 0mmmol) and stirred for 30mins.The mixture was stirred and gently refluxed at room temperature for 8 hours. The ethanol was evaporated under rotavapor and the resulting yellow solid was recrystallised with dichloromethane. Yield: 80%

Mp<180°C $C_{43}H_{54}O_2N_2$ (630.9)

^{1}H NMR ($CDCl_3$): δ (phenolic OH) 13.636(s, 2H) ;): δ 8.38-8.36(CH=N) δ(Aromatic) 7.2(d,2H);7.4(d,2H); δ 7.4-7.2(m,8H) ;4.02(S,2H); δ 1.29 -1.34 (S,18H) (t-butyl)

Preparation of the complex Fe_2 $(L1)_2$:

To a 20 ml of DMF solution of the ligand (0.136g,1.0mmol), triethylammine (0.2g, 280µml, 2.0mmol) was added to abstract the phenolic hydrogen of the ligand added solution of Ferric chloride(0.0324,1.0mmol) dissolved in DMF (20ml).The resulting dark greenish solution was refluxed for 4 hours and whereby,it changed red solid. Red precipitate was obtained on evaporation of the solution at room temperature for several days. The complex was recrystallised from Petroleum ether.

Mp<180°C $C_{86}H_{104}O_4N_4Cl_2.Fe_2.(2H_20)$-(1494.9)

Synthesis of the ligand L2:

Synthesis of [N, N -4"-phenoxy–bis(3,5-di-tert-butylsalicylideneimine)] – benzene

3, 5 di t- butyl-2-hydroxybenzaldehyde (0.468, 2. 0mmmol) in ethanol (100ml) was mixed with 4, 4 oxydianiline (0.2g 1.0mmmol) and stirred for 30mins. The mixture was stirred and gently refluxed at room temperature for 8 hours. The ethanol was evaporated under rotavapor and the resulting shining dark yellow flakes was recrystallised with dichloromethane.

Yield: 80%

Mp<180°C $C_{42}H_{52}O_3N_2$ (632.89)

^{1}H NMR ($CDCl_3$): δ (phenolic OH) 13.6(s, 2H); δ 8.36-8.39(CH=N) δ (AROMATIC) 7.18(d, 2H) ;6.68 (d,2H); δ 6.9-7.2(m,8H) ; δ 1.29 -1.34(S, 18H) (t-butyl)

Preparation of the complexesFe_2 $(L2)_2$:

To a 20 ml of the DMF solution ligand(0.131g,1.0mmol), triethylammine (0.2g, 280µml, 2.0mmol) was added to abstract the phenolic hydrogen of the ligand, followed by a solution of Ferric chloride(0.034g.1.0mmol) dissolved in DMF (20ml).The resulting dark greenish solution was refluxed for 4 hours and whereby, it changes to red solid. Red precipitate was obtained on evaporation of the solution at room temperature for several days. The complex was recrystallised from Petroleum ether.

Mp<180° $C_{84}H_{100}O6N_4Cl_2.Fe_2.(2H_20)$-1498.9

Synthesis of ligand L3:

1, 3- bis [3'-aza-4'-(5"-naphthyl 2"-hydroxyphenyl -1-ol]-propane 1, 3diamine

A substance of 1,3 diamino 2-hydroxy propane (0.09012g (1mmol) was added in 100 ml of dichloromethane to the solution of 2-hydroxy naphthaldehyde 0.344g (2mmol) at room temperature. The white solution stirred for 30 minutes. A yellow product was isolated. The product was collected by filtration, washed with minimum volume of dichloromethane and dried in vacuo P_4O_{10} (89%) .

Mp<150 $C_{25}H_{22}O_3N_2$ (398.4589)

^{1}H NMR (CDCl3): δ (phenolicOH) 14.4(s,2H); (Aromatic hydrogen) 9.21-6.93(8H); (Allylic OH) 4.84(s, 1H); 3.73 (t,4H); 2.76(s,1H); **2**.66(s,2H)

Synthesis of ligand L4:

Synthesis of (1,3 bis –[3'-aza-4'-(5"-napthyl-2"-hydroxypheny)-prop-

4-en-1-yl)]-ethane 1,2-diamine(H2L)

A solution of Triethylenetetramine (0.1462g (1mmol) was added in 100 ml of dichloromethane to the solution of 2-hydroxy naphthaldehytde 0.344g 2mmol) at room temperature. The red colour solution stirred for 30 minutes. A yellow product was isolated. The product was collected by filtration washed with minimum volume of dichloromethane and dried in vacuum P_4O_{10} Mp<150 $C_{28}H_{30}O_2N_4$ (454.5)

^{1}H NMR (CDCl3): δ (phenolicOH) 14.4(s,2H); (Aromatic hydrogen) 8.71-7.19(8H); (Allylic OH) 3.64(t, 4H); 2.74 (t,4H); 2.75(t,4 H); 2.16(s,2H)

Preparation of complexes (III&IV)

The complexes were synthesized by addition of $FeCl_3.5H_20$ (0.0324g/1mmol) in 20mL of DMF to a solution of Schiff base (1mmol ligand in 20 ml DMF) in the presence of Et_3NH (0.2ml, 2mmol) and the reaction mixture was heated under reflux for 2 hrs. The volume of the obtained solution was reduced to one-half by evaporation. The obtained red solid powdered material of both complexes (III &IV) were filtered washed with absolute acetonitrile and placed in the desiccator for two days.

Screening of antimicrobial activity

Test microorganisms

Klebsiella pneumoniae, Salmonella typhi

Preparation of discs

Known quantity of extracts or fractions was dissolved in DMSO: methanol of 1:1 ratio. This in turn was diluted with equal volume of Phosphate Buffered Saline (PBS pH7).It was then filter sterilized by making use of sortorious syringe filter of pore size 0.22µm. sterile discs of 6mm diameter (Himedia) were loaded with various concentration of extract and fractions and were dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and used as negative control. Oxytetracyline loaded Hi-Media discs were used as positive control.

Preparation of inoculum

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at37°C for 4 hours in a shaker (Orbitech, Scigenics, India) and were used for anti-bacterial test and to look for the MIC ofvarious extracts and fractions.

Determination of Antibacterial activity

Disc Diffusion method was followed (Bauer et al. 1996)[21] to determine the antibacterial activity of various extracts and fractions. Petriplates containing 20ml of Mueller Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains. By making use of template drawn extracts and fractions loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms. Oxytetracyline antibiotic disc obtained from M/s Hi-Media Laboratories Ltd, Mumbai was used as a positive control and solvent loaded discs were used as negative control. This was incubated at 37°C for 24 hours in an incubator (Rands SBC).The test was

performed intriplicates. The zone of inhibition was measured by making use of Antibiotic Zone scale (Hi –media)

Determination of Minimum Inhibitory Concentration

NCCLS for disc diffusion method and agar dilution technique for MIC testing. Stock solution of various plant extract was prepared by making use of DMSO: methanol in the ratio of 1:1 which in turn was diluted with equal volume of phosphate buffered saline, pH 7. Mueller Hinton agar was prepared, sterilized and kept ready in molten condition. 20ml of the molten media was taken and was mixed with known concentration of different extracts/fractions and were added in different tubes. This mixture was swirled carefully for complete mixing of extract and media and poured on to the plate. After getting solidified it was inoculated with the test organism and standard organism. The plates were incubated at 37°C for 24 hours. MIC was recorded on the growth of the organisms.

Results and Discussions

The results obtained from the study are presented in Table 1 and Table 2. The compound $Fe(NHAD)Cl_2$ at the dose of 100µg/ml showed 75% antibacterial activity against the Gram positive bacteria *S. pyogens*, 100% against *Micrococcus luteus*, *Lactobacillus* sp and *Bacillus subtilis* (13-22mm inhibitory zones) but only 50% against the five Gram negative organisms used in this study (13-16mm inhibitory zones). Fe $(NHTEN)Cl_2$ at the dose of 100µg/ml was effective against two Gram positive (ie) *Lactobacillus* sp and *S. aureus*) (14-21mm inhibitory zones) and three Gram negative bacteria(13-15mm inhibitory zones). Fe_2 $(TBSHOD)Cl_2$ with the same dose of 100µg/ml exhibited antibacterial activity against all Gram–positive bacteria (12-22mm inhibitory zones) and four Gram–negative bacteria (12-14mm inhibitory zones). $Fe_2(TBSMD)Cl_2$ with similar dose as earlier mentioned showed a pronounced effect on all Gram positive bacteria(9-20mm inhibitory zones) and four Gram negative bacteria(10-14mm inhibitory zones). Most of the bacterial strains used as test system in our investigations showed high susceptibility to the broad spectrum antibiotic oxytetracyline. Mononuclear and Binuclear ligands and complexes were synthesized and subjected to antibacterial susceptibility test against both Gram positive and Gram negative pathogens using disc diffusion and drug dilution method. L1 and L2 were the dinuclear ligands, L3 and L4 were the mononuclear ligands similarly C1 and C2 were dinuclear complexes and C3 and C4 were mononuclear complexes. Antimicrobial activities of four different ligands revealed that more significant activity was exhibited by L3 against Lactobacillus sp. (18mm) (Table 1) followed by L4 against *Aeromonas* sp., (16mm). The pathogens like *Shigella dysenteriae* (10mm by L4 & L3and 11mm each by L1 & L2), Bacillus subtilis (13mm by L2 & L3 and 12mm each by L4 & L1); *Pseudomonas aeruginosa* (12mm each by L1, L3, L4& L2); Streptococcus pyogenes (12mm by L3; 13mm by L2; 11 mm by L4 & 9mm by L1); *Klebsiella pneumoniae* (13mm by L1, 11mm L3 and 7mm by L4 &12mm by L2); *Streptococcus faecalis* (12mm by L1; 10mm by L3 and 9mm by L4 & 11mm by L2) were effectively inhibited by all the ligands tested at 100 µ g/disc concentrations. When these ligands were complexed with Fe ions showed better inhibitory effect against all the pathogens tested at 100µ g/disc concentrations (Table 1). C3 has been considered as the ideal complex, which showed better activity against all the pathogens tested at 100 µg/disc concentrations. Zone of inhibition produced by the C3 complex ranges from10mm to 22mm. Though other complexes produced higher zone of inhibition against any one of the organism (C1 produced 20mm against *Aeromonas* sp., C3 produced 22mm against Lactobacilli sp., and C4 produced 21mm against *Lactobacilli* sp.,), they didn't exhibited inhibitory activity against important gram positive and gram-negative pathogens (C1-*Micrococcus luteus*; C3-Enterobacter aerogenes and C4 – *Salmonella paratyphi*).

Table 1: Inhibitory effect of pathogenic bacteria in the agar-diffusion method for ligands&Complxesin mm at 100µg/ml

Microrganisms	L1	L2	L3	L4	C1	C2	C3	C4
Escherichia coli	9	10	12	11	14	12	16	13
Salmonella Typhi	11	0	9	10	12	11	13	12
Shigella Dysenteriae	11	11	10	10	14	17	14	12
Pseudomonas aeruginosa	12	12	12	12	11	15	13	16
Aeromonas sp	10	11	12	16	20	16	12	10
Lactobacillus sp.	15	13	18	9	12	20	22	21
Staphylococcus aureus	9	11	11	10	13	13	12	14
Bacillus subtilis	12	13	13	12	12	14	16	15
Streptococcus pyogens	9	13	12	11	14	13	14	10
Enterobacter aerogens	10	12	0	10	10	14	20	15
Klebsiella pneumoniae	13	12	11	7	14	14	15	10
Micrococcus luteus	7	0	13	0	0	13	17	10
Salmonella paratyphi	0	12	9	0	12	10	13	0
Streptococcus facecalis	12	11	10	9	12	13	15	12

Figure:2

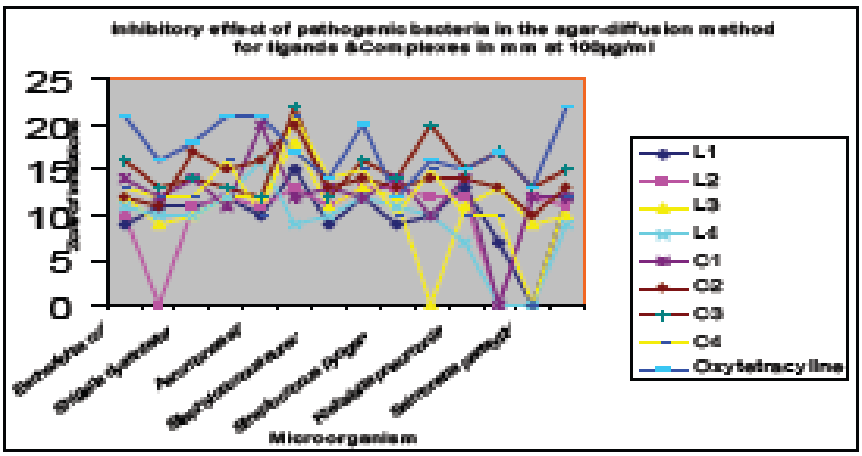
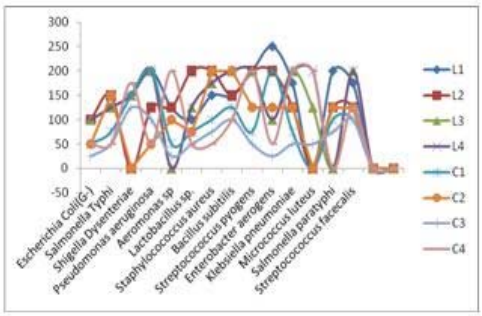


Table 2: Minimum Inhibitory effect of pathogenic bacteria in the agar-diffusion method for ligands and Complexes

Microrganisms	L1	L2	L3	L4	C1	C2	C3	C4
Escherichia coli	100	100	100	100	50	50	25	50
Salmonella Typhi	125	150	125	125	75	150	50	50
Shigella Dysenteriae	150	---	150	150	150	--	125	175
Pseudomonas aeruginosa	200	125	200	200	200	50	100	50
Aeromonas sp	125	125	---	---	50	100	25	200
Lactobacillus sp.	100	200	125	125	75	75	50	50
Staphylococcus aureus	150	200	175	175	100	200	75	50
Bacillus subitilis	150	150	200	200	125	200	100	100
Streptococcus pyogens	200	200	200	200	75	125	50	200
Enterobacter aerogens	250	200	100	100	200	125	25	50
Klebsiella pneumoniae	175	125	200	200	75	125	50	200
Micrococcus luteus	---	----	125	200	---	----	50	200
Salmonella paratyphi	200	125	--	--	100	125	75	--
Streptococcus facecalis	175	125	200	200	100	125	100	125

Figure 3: Minimum Inhibitory effect of pathogenic bacteria in the agar-diffusion method for ligands and Complexes in mm at 100µg/ml:



Results of Table 1 and 2 evidenced that complexes were showed better antimicrobial potentials than the ligands. Average zone of inhibitions obtained using complexes were much higher when compared to ligands. C2 complex yielded zone of inhibition ranges from 10mm to 20mm whereas L2 ligand yielded 0 – 13mm zone of inhibition. Average zone of inhibition of complexes were C1-12.1mm; C3-15.7mm; C4-12.7mm & C2-14mm. Ligands produced variable and lower zone of inhibition, which were L1- 9.5mm; L3-10.8mm; L4-9.5mm and L2-10mm. The establishments of the minimum inhibitory concentrations (MICs) of antimicrobial means are a more precise method for determination of their effect. MIC effect of both ligands and complexes were performed by adopting standard protocol called Drug dilution method. Ligands inhibited the growth of gram positive and gram- negative pathogenic bacteria and concentration required to inhibits the growth ranges from 100µ g/ml concentrations to more than 1000 µ g/ml concentrations (Table 2). Complexes also prevent the growth of bacteria, its concentration ranges from 25 to 250 µ g/ml concentrations (Table 2). Here also complexes showed better activity when compared to ligands. C3 inhibited pathogenic bacteria like *E. coli, Aeromonas* sp., and *Enterobacter* at 25 µ g/ml concentrations whereas the same bacteria were inhibited by the ligands at higher concentrations (*E. coli* - 100 δ g/ml, *Aeromonas* sp., - 100 µ g/ml and *Enterobacter* at 75 µ g/ml concentrations). Mononuclear complexes and ligands showed slightly better antimicrobial activity against all the pathogens tested.

Among the compounds tested Fe_2 (TBSHMD) (C2) was found to be most active with the lowest MIC against the gram positive bacteria .The highest MICs were established for Fe (NHAD) Cl_2 (C3). The sensitivity of Gam – positive bacteria to Fe(NHAD)Cl_2 were slightly higher as compared to Fe(NHTEN)Cl_2 (C4)and lower than those to Fe $_2$(TBSHMD)Cl_2 but the difference were not significant. For most Gram-positive bacteria tested the MIC's established by four iron complexes were mostly similar. For, *Lactobacillus* sp, and *Pseudomonas aeruginosa*. The MICs were lower for Fe_2 (TBSHMD) Cl_2 than those of other two iron complexes. On the other hand Fe (NHAD) Cl_2 showed highest activity against *E. coli* and *E. aerogens*. The control solution PBS containing the same concentration of DMSO as samples examined when tested independently by both methods. The results obtained showed that the bacterial strains used in the experiments were sensitive to iron complexes investigated in spite of that their ligands.

Conclusions

According to Okama *et al* (1990)[22] the antimicrobial activity of the drugs increased in a low iron environment and decreased in the presence of a high iron concentration (occurrence for the site penetration-receptors). Many pathogenic bacteria receive their essential iron by assimilation through cell surface receptors (Diarra *et al*., 1996). Probably the bacteria assimilate better iron compounds than the copper ones due to use of haeme uptake mechanism.The antimicrobial activity of iron complexes with ligands containing catechol moiety N,N bis(naphthy 2-hydroxy) 1,3 diamminopropane (NHDAP);(C-3) N,N bis (naphthy 2-hydroxy) triethylenetetrammine (NHTEN);(C-4) N,N bis (t-Butylsalicyl 2hydroxy) 4,4"diamminodiphenylmethane (TBSHMD); (C-2) N,N bis (t-Butylsalicyl-2-hydroxy) 4,4"oxydianiline -(TBSHOD)(C-1). The examined mononuclear iron complex has more or less pronounced effect in Gram positive and Gram negative strains as compared to that of dinuclear complex. The imperfect solubility of the dinuclear iron complexes could be considered as one of the constrain for obtaining much better antibacterial activity when compared to mononuclear complexes.The examined mononuclear iron complexes has more or lesspronounced effect in gram positive and gram negative strains ascompared to that of dinuclear complex. The examined ironcomplexes manifest inhibitory activity against gram positive andgram negative bacterial strains in the experiments. The complexesof Fe (III) with (NHAD) &, (NHTEN), Diiron with (TBSHMD) are moreactive than that of dinuclear complexes (TBSHOD).The dinuclearcomplexes of (TBSHOD) has less active compared to others becauseof the presence of oxydianiline which hinder the activity. The schiffsbases, which were inactive before complex become active and less active one.

References:

1.Warhurst., A. M. and Fewson., C. A. (1994) Crit. Rev. Biotechnol. 14, 29-73

2.Bouwer, E., J. and Zehnder., A., J. (1993) Trends Biotechnol..11, 360-67.

3.Gibson., D. T. (1968) Science. 161, 1093-1097.

4.Nozaki, M. (1979) Top. Curr. Chem. 78, 145-186.

5.Nakai, C., Horiike, K., Kuramitsu, S., Kagamiyama, H., and Nozaki, M. (1990) J. Biol. Chem. 265, 660-665.

6.Neidle, E. L., Hartnett., C., Bonitz, S. and Ornston, N. L. (1988) J. Bacteriol. 17, 4874-4879

7.Eck, R., and Belter, J. (1993) Gene 123, 87-92

8.Dorn, E., and Knackmuss, H.-J. (1978) Biochem. J. 175, 73-84

9.Fujiwara, M., Golovleva, L. A., Saeki, Y., Nozaki, M. and Hayaishi, O. (1975) J.Biolog.Chem. 250, 4848-4852

10.Broderick, J. and O'Halloran, T. V. (1991) Biochemistry 30, 7349-7358

11.Nora.H.Ala-Sha'alam.,Molecules.2007,12(5),1080-1091

12.Burger R.,M.Bull.chem.Soc.Ethiop.2010,24(2).199.20

13.Ali-Shiri ASM and Abdal-Fattah HM.J Therm Anal Cal.,2003,71,643.

14.Elzahany EA., Hegab,KH., Khabil, SKH and Youssef NS,Aust J Basic Appl Sci.,2008, 2(2),210.

15. Turk. J. Chem .32(2008), 487-493.

16 *Murakami* Y, Uemura K, Sudo T, Hayashidani Y, Hashimoto Y, Nakagawa N, *et al*. Am J Surg 2008; 195:757-62.

17.Diarra, M. S., M. C. Lavoie, M. Jacques, I .Darwish, E. K. Dolence, J. A. Dolence, AGhosh, M.Ghosh, M. J. Miller & F. Malouin, 1996. Species selectivity of new siderophore-drug conjugates that use spe- cific iron uptake for entry into bacteria Antimicrobial Agents and Chemotherapy, 40, No 11, 2610–2617.

18.Gvozdyak, R. I., T. M. Shvets, N. F. Ku- shchevskaya & R. O. Denis, 1996. The antibacterial activity of pre parations with highly dispersed iron. Mikrobiolohichnyi Zhurnal (Kiev), 58, No 6, 45–49.

19.Bacchi, A., M. Carcelli, P. Pelagatti, C. Pe-lizzi, G.Pelizzi & F. Zani, 1999. Antimicrobial and mutagenic activit y of some carbono- and thiocarbonohydrazone ligands and their copper (II), iron (II) and zinc (II) complexes. Journal of Inorganic Biochemistry, 75, No 2, 123–133.

20.Donde, K. J., V. R. Patil & S. P. Malve, 2003. Antimicrobial studies of hydrazone complexes of Hg (II) and Fe (II) divalent metal ions. Acta Poloniae Pharmaceutica, (2003).60, No 3, 173–175.

21.Bauer, A.W., W.M.M. Kirbey, J.C. Sherries and M. Truck: Antibiotic susceptibility testing by standardized si ngle disc method. Am. J. Clin. Pathol., 45: 493-496 (1996). .

22.Okamoto, R., T. Hara, T. Yoshida, Y. Orikasa, H. Ogino, K. Iwamatsu & S. Inouye, 1990. In vitro antimicrobial activity of a novel aminothiazolylglycylcephalosporin, MT0703S, compared with that of cef- tazidime, cefoperazone and aztreonam. Drugs under Experimental and Clinical Research, 16, No 4, 157–165.