Pak. J. Chem. 3(4): 177-181, 2013 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2013.v03.i04.p06

Antitumor Activity of New Quinoxaline Analogues and Its Complexes

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ABSTRACT
This study shows synthesis of a new quinoxaline derivatives (LH) and preparation of copper (Cu-LH) and cobalt (Co-LH) complexes and studying the effect of these compounds on the activity of lactate dehydrogenase (LDH) a primary enzyme to the process of anaerobic glycolysis which is the source of energy in tumor cell. This study included of 40 women with recently diagnosed of malignancies Breast cancer; (20) subject women in stage I&(20)subject women in stage II who were admitted to Medical City Hospital with age ranged (37-45) years and 30 apparently healthy woman matched for age served as controls.
Serum LDH activity was assayed on the time of the diagnosis in all cases and after addition of different concentrations (10^{-1}, 10^{-2}, 10^{-3}) of quinoxalin derivatives and transition metals complexes *in vitro*. The results revealed an inhibitory effect of LH and its complexes with Cu and Co in all concentrations used in this study on LDH activity. The inhibition percent's increased with increase concentration for LH and its complexes. Also the results reveal that the inhibitory effect of LH in all concentrations used were more than inhibitory effect of its complexes. Moreover the inhibitory effect of LH-Co complex was more than inhibitory effect of LH-Cu complex in all concentrations used. The inhibition ability of quinoxaline derivative and its complexes on the enzyme lactate dehydrogenase leads to the fact that these compounds can be characterized as an active anti –cancer substances for one of the metabolic enzymes necessary to feed the cancer cells.

Keyword: breast cancer, quinoxaline derivatives, copper and cobalt complexes, lactate dehydrogenase

1. INTRODUCTION
Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. There are many different types of breast cancer, with different stages (spread), aggressiveness, and genetic makeup; survival varies greatly depending on those factors. The disease occurs almost entirely in women, but men can get it, too[1].

For the early detection of carcinoma of various origins, a number of biochemical markers have been studied to evaluate the malignancy. Tumor associated markers reflect behavioral changes from tissue to blood, resulting in changes in levels of enzymes, proteins and hormones both in cancerous tissue and blood because of unchecked proliferation of cells. Therefore, alteration in particular enzyme contents in serum could be a good index of malignancy in its early and best manageable stage[2]. A relationship between neoplasia and increased LDH levels has been reported by many worker's in both human and animal tumors[3] also (Maity C,etal 1988)[4] reported the Serum levels of glycolytic enzymes were found to be increased in patients with breast carcinoma. Thus LDH levels are a good adjunct in the diagnosis, are an indicator of the stage of the disease, response to treatment and prognosis of the patient [5].

LDH is distributed widely in body tissues and is raised in variety of physiological and pathological status. Raised levels of LDH are seen in malignancies because of high rate of glycolysis, increased production of enzyme by tumor cells, change in the permeability of cells, allowing leakage of soluble enzyme into circulation and because of tumor blockade of the duct system through which enzyme passes[6].

Lactate dehydrogenase is an oxidation reduction enzyme which reversibly catalyses the reaction between pyruvic acid to lactic acid. LDH is a tetrameric enzyme, containing 2 major subunits (A and B) coded by 2 different genes (LDH-A and LDH-B), which may form 5 isozymes[7].

All 5 isozymes can catalyze the forward and backward conversion of pyruvate and lactate. LDH-A (LDH-5, M-LDH, or A4) kinetically favors the conversion of pyruvate to lactate whereas LDH-B (LDH-1, H-LDH, or B4) predominantly converts lactate to pyruvate, which will be further oxidized through the TCA cycle[8].

The LDH-A and LDH-B subunits and their ratio are very important in the formation and function of the tetrameric enzyme, and the subunit composition impacts on the kinetics and the direction of the LDH-regulated reaction. In cancer patients, serum total lactate dehydrogenase (LDH) levels are often increased, and the gene for LDH-A protein is often upregulated in tumors[9]. Because LDH-A protein is required for the maintenance and progression of many tumors[10], it is also becoming a potential target for cancer therapy[11].

Quinoxaline derivatives are double nitrogen containing heterocyclic compounds which possess biological importance. A wide variety of pharmacological properties has been associated with quinoxaline derivatives. These include anticancer[12], antidiabetic[13], anti-inflammatory[14] antimicrobial[15] and antivira[16] activities.

The aim of this study to synthesize certain new quinoxaline analogues and to prepare transition metal complexes to be evaluated for antitumor activity Through studying its impact on the activity of lactate dehydrogenase enzyme a primary enzyme to the process of anaerobic glycolysis, which is a source of energy in tumor cell.

*Corresponding Author Received 15th November 2013, Accepted 15th December 2013

2. MATERIAL AND METHODS

2.1 *Subject*

Forty women with recently diagnosed of malignancies Breast cancer; (20)subject women in stage I and (20)subject women in stage II who were admitted to Medical City Hospital with age ranged (37-45) years and 30 apparently healthy woman of matched age served as controls.

2.2 *Enzyme assay*

Serum LDH activity was assayed according to the method of (Wroblewsk F, el.al 1955)[17] by using randox kit (England). The determination was done on the time of the diagnosis in all cases and after addition of quinoxalin derivatives and transition metals complexes *in vitro*

2.3 *Synthesis of 3-amino quinoxaline -2 (1H)-thione and its complexes*

2.3.1 *3-amino quinoxaline -2 (1H)-thione (LH)*

0.240 gm. portion of dithio-oxamide were dissolved in 10 ml of ethanol. The solution was refluxed at 70C°, 0.216gm 1, 2 phenylene diamine in 10ml of ethanol were added. Heating was stopped after half an hour.

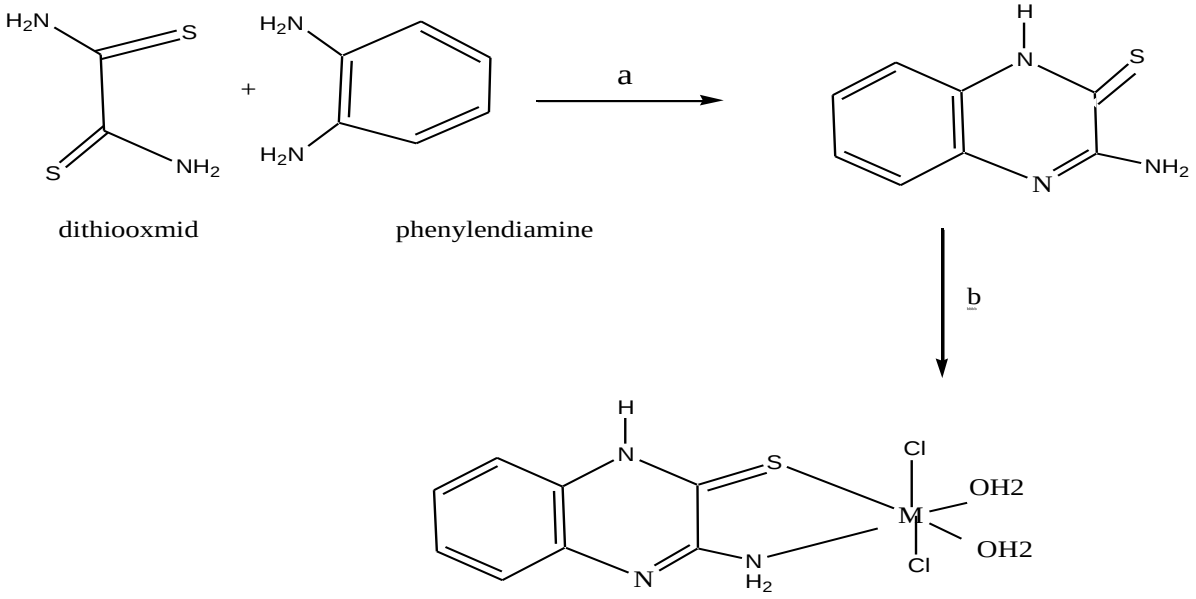
The mixture continue refluxed for 8hrs at room temperature for 30min then allowed to stand for crystal formation, filtered, washed with water and recrystallized from hot (0.4bezen+1. 6CCl4).

The compound obtained was yellowish-brown solid (46%) confirmed by ; **IR (KBr)in cm^{-1} :** (3500,3400) NH_2, (3294)NH,(3080w)CH aromatic, (1585st)C=N+C=C, (1153m)C=S; **^{1}HNMR(DMOS) in ppm :** (2.5) NH_2,(4.3)NH, (6.5-7)H aromatic; **C^{13}NMR(ppm) :** (150-160)C-NH_2, (135-115)C-aromatic, (194)C=S; **UV-Vis(nm):** (242,420). Analysis calculated for $C_8H_7N_3S$, (177)M.Wt(53.7)C,(3.7)H,(23.38)N, found(54.02)C,(3.95)H,(23.7)N,m.p(178-180)C^0.

2.3.2 *Metal complexes for (LH)*

Cu-LH & Co-LH– complexes: to(LH)solution (0.177gm)in 5ml ethanol were added (0.211gm)$CuCl_2.6H_2O$ in ethanol solvent, the reaction was allowed to stir for (1hr), dark brown solid was formed, the product was washed with water, recrystllized with hot ethanol. similar method was used to prepare LH-Co-complex except $CuCl_2.6H_2O$ was replaced by solution of (0.208gm)$CoCl_2.6H_2O$, the complexes were obtained confirmed by;**IR (KBr) cm^{-1}for LH-Cu ;LH-Co complexes respectively :** (3150,3240w), (3200,3250w) NH_2, (1157, 1157m) C=S, (3080-3450 br), (3040-3380br), (763,760ben)-H_2O, (520,530)M-N ;**UV -Vis:** (630)nm for LH-Cu, (605,675), LH- Co in absence LDH,(495,540)nm in presence LDH.

Analysis calculated for : $C_8H_{11}N_3$ S O_2Cl_2Cu, $C_8H_{11}N_3$ S O_2Cl_2Co, (348,345)M.wt, (27.6,27.09)C,(3.16,3.2)H,(12.06,12.24)N,(9.1,9.3)Sfound(27.46,27.61)C,(3.01,3.0)H,(12.01,12.0)N m.p(225,215), molar conductivity measurement is non electrolyte, molar ratio M:L 1:1, Yield dark-brown solid 50%, brown solid 54%, in all cases from result the structural formula were proposed complexes are octahedral geometry[(1)] as shown in fallowing of scheme.



Conditions: (a) ethanol, reflex 1(70 C ^{0}, ½ hour), reflex 2,(8hour at R.T),(b)ethanol, reflex (1hour at R.T),M=$CoCl_2.6H_2O$,$CuCl_2.6H_2O$

2.3.3 *Preparation LH and metal complexes solution*

10ml of different concentrations (10^{-1}, 10^{-2}, 10^{-3}) M of each of LH and its complexes were prepared, are shown in table(1).

Table.1: The molecular formula and weight of LH and its complexes for preparation of stock solutions

Formula	Wight (gm)	Solvent (H_2O: ethanol)
$C_8H_7N_3S$	177	1:9
$[C_8H_{11}N_3SO_2Cl_2Cu]$	348	1:9
$[C_8H_{11}N_3SO_2Cl_2Co]$	345	1:9

3. RESULTS AND DISCUSSION

Table.2: LDH activity without inhibitor and the inhibition percentage of LH in different concentration on LDH in serum of stages I&II of breast cancer patients

	Stage I		Stage II	
	LDH activity(u/l)	inhibition%	LDH activity(u/l)	inhibition%
without inhibiter	388		507	
with 10^{-1} M of LH	222.8	34	330	35
with 10^{-2}M of LH	299.2	11.48	442	12.9
with 10^{-3} M of LH	328.4	2.85	490	3.4

Table.3: LDH activity without inhibitor and the inhibition percentage of LH -Cu in different concentration on LDH in serum of stages I&II of breast cancer patients

	Stage I		Stage II	
	LDH activity(u/l)	inhibition%	LDH activity(u/l)	inhibition%
without inhibiter	388		507	
with 10^{-1} M of LH –Cu	296	12.43	442	12.83
with 10^{-2}M of LH-Cu	311.2	7.93	465.1	8.27
with 10^{-3} M of LH-Cu	312.8	7.46	470	7.3

Table.4: LDH activity without inhibitor and the inhibition percentage of LH-Co in different concentration on LDH in serum of stages I&II of breast cancer patients

	Stage I		Stage II	
	LDH activity(u/l)	inhibition %	LDH activity(u/l)	inhibition %
without inhibiter	388		507	
with 10^{-1} M of LH-Co	236.4	30.1	350	30.97
with 10^{-2}M of LH- Co	303.2	10.3	450	11.75
with 10^{-3} M of LH- Co	308.4	7.76	462	8.88

Data in the tables (2), (3) and (4) shows the inhibition percentages of LH and its complexes in different concentration 10^{-1},10^{-2},10^{-3} respectively on LDH activity in sera of stage I&II of Brest cancer. The results revealed an inhibitory effect of LH and complexes with Cu and Co in all concentrations used in this study on LDH activity and the inhibition percentages were increased with concentration increase for LH and its complexes. Also these tables show the inhibitory effects of LH in all concentrations used more than inhibitory effects for its complexes and the inhibitory effects of LH-Co complex were more than inhibitory effects of LH-Cu complex in all concentrations used.

Cancer is caused by abnormalities in the genetic material of the affected cells. On the way to tumorigenesis there occurs an accumulation of successive mutations in proto-oncogenes and suppressor genes that deregulates the cell cycle. The events key to tumorigenesis are for instance point mutations in DNA sequences, chromosomal aberrations such as translocations or deletions and changes that affect the chromatin structure such as methylation of DNA or acetylation of histones. Cancer therapy is mostly based largely on surgery, radiotherapy, hormone and chemotherapy [18].

The word 'chemotherapy' was first introduced by the German chemist and immunologist Paul Ehrlich, which means treatment of the diseases with chemicals. Chemotherapeutical drugs aim at killing malignant tumor cells more or less selectively. Throughout the years, there were many key advances in the development of cancer chemotherapy, beginning in the first half of the 20th century[19].

The first chemotherapeutic agents developed—nucleoside analogues referred to as antimetabolites— target nucleotide biosynthesis through the direct inhibition of enzymes used in DNA synthesis[20]. Another therapeutic

opportunity that has been explored is the small-molecule inhibition of key enzymes involved in metabolic pathways such as glycolysis and fatty acid synthesis[21].

The inhibitory abilities of quinoxaline derivatives and its complexes show the effectiveness of the lactate dehydrogenase enzyme, which lead to the fact that it can be characterized as anti-cancerous activity so as to their ability inhibitory to one of the metabolic enzymes necessary to feed the cancer cells.

The inhibitory effect of quinoxaline derivatives on lactate dehydrogenase activity was believed to be due to that quinoxaline derivatives are double nitrogen containing heterocyclic compounds were interchelators bind to DNA of LDH-A gene by non-covalent interactions and constitute DNA–interchelator complex. The only recognized forces that maintain the stability of the DNA–interchelator complex, even more than that of DNA alone, are van der Waals, hydrogen bonding, polarization and hydrophobic forces[22]. The force of interaction between compound and DNA usually correlates with the anticancer activity[23].

LDH-A gene that encodes for LDH-A protein synthesis one of the subunits of LDH is the key enzyme of anaerobic glycolysis, which is the main source of energy in cancer cells according to Warburg's hypothesis was postulated by the Nobel laureate Otto Heinrich Warburg in 1924[24]. He hypothesized that cancer, malignant growth, and tumor growth are caused by the fact that tumor cells mainly generate energy (as e.g. adenosine triphosphate / ATP) by non-oxidative breakdown of glucose (a process called glycolysis). This is in contrast to "healthy" cells which mainly generate energy from oxidative breakdown of pyruvate. Pyruvate is an end-product of glycolysis, and is oxidized within the mitochondria. Hence, according to Warburg, the driver of cancer cells should be interpreted as stemming from a lowering of mitochondrial respiration. Warburg Effect would be defined as the observation that cancer cells exhibit glycolysis with lactate secretion and mitochondrial respiration even in the presence of oxygen[25].
In the recent years much effort has been made to increase the number of therapeutic metal complexes. Historically, metals and metal complexes have played a key role in the development of pharmacy and modern chemotherapy. However, they still remain a tiny minority of all therapeutics on the market today[26].

Transition metal complexes that are suitable for binding and cleaving double-stranded DNA are of considerable current interest due to their various applications in nucleic acid chemistry like foot-printing and sequence-specific binding agents, for modelling the restriction enzymes in genomic research, and as structural probes for therapeutic applications in cancer treatment. Cleavage of DNA can be achieved by targeting its basic constituents like base and/or sugar by an oxidative pathway or by hydrolysis of phosphoester linkages. Copper complex are known to be useful for oxidative cleavage of DNA involving nucleobase oxidation and/or degradation of sugar by abstraction of deoxyribose hydrogen atom(s).

The ability of the LH-Cu inhibitory to the effectiveness of the enzyme lactate dehydrogenase as possible be interpreted on the basis that complexes containing strong Lewis acids like copper (II) is suitable for hydrolytic cleavage of DNA[27].

The results of this study agree with Sigman and co-workers who have reported *bis* (phen) copper (I) complex as the first copper-based "chemical nuclease" that cleaves DNA in the presence of H_2O_2 and a thiol[28].

The cobalt complexes are of more limited medical usage compared to copper complexes, Since the first reported studies on the biological activity of cobalt complexes in 1952[29,] Cobalt(III) complexes have been widely studied as anticancer agents[30].

Co^{II} is stable in aqueous solution, in strong field Co^{II} is oxidation to Co^{III} [31]. From our results Uv-Vis for LH-Co complex in absence of LD H (605,675) nm due[4] $T_1g \xrightarrow{V_2} T_2g(F)$, $^4T_1g \xrightarrow{V_3} {}^4A_2g(p)$[32] in presence of LDH the spectrum of compound shows an increase in the intensity and change in shape, two strong transitions in (495,540)nm due to $^1A_{1g} \xrightarrow{V_1} T_{2g}$, $^1A_{1g} \xrightarrow{V_2} T_{1g}$ that was showed the oxidation of Co^{II} to Co^{III} in more strong felid[33].

The Co (III) carrier is used as a transporter for the drug and the activation by a bioreductive pathway to the Co (II) complex which releases the inhibitor ligand intracellular[34]. Cobalt (III) complexes have been described as hypoxia selective antitumor agents the concept of such a design is based on the fact that the tumor cells develop resistance to chemotherapeutic agents under anaerobic conditions. They may be reduced under hypoxic conditions to Co (II) species followed by loss of neutral ligand[35-36]. Through the progress it can be explained the high inhibitory ability of cobalt complex (LH-Co) compared with the copper complex (LH-Cu) of the effectiveness of anaerobic glycolysis enzyme LDH

4. CONCLUSION

The inhibition ability of quinoxaline derivative and the high inhibitory ability of cobalt complex (LH-Co) compared with the copper complex (LH-Cu) of the effectiveness of anaerobic glycolysis enzyme LDH leads to the fact that these compounds can be characterized as an active anti –cancer substances for one of the metabolic enzymes necessary to feed the cancer cells.

5. REFERENCE

1. Simpson, J. F. and Wilkinson, E. J., In: Bland KI, Copeland EM, The breast: comprehensive management of benign and malignant disorders. 3rd Ed. St Louis: Saunders; **(2004)**.

2. Chandrakanth, K. H., Jayaprakash, N., Murthy, D. S., Satishkumar, D., and Anand Pyati, Int J Pharm. Biosci **(2011)** 2: Issue 4, 489-498.
3. Starkweather, W. H., Scoch, H. K. M., Biochem Biophys Acta **(1962)** 62, 440-2, http://dx.doi.org/10.1016/0006-3002(62)90281-0.
4. Maity, C., Roy, M., Maity, C. R., Indian journal of surgery **(1988)** 323- 329.
5. Kher, A., Moghe, G., Deshpande, A., Indian J Pathol Microbiol **(1997)** 40(3), 321-326.
6. Talageri, V. R., Ind J Cancer, **(1977)** 14,50- 57
7. Everse, J., Kaplan, N. O., Adv Enzymol Relat Areas Mol Biol **(1973)** 37,61–133
8. Stambaugh, R., Post, D., J Biol Chem **(1966)** 241, 1462–7.
9. Fantin, V. R., St-Pierre, J., Leder, P., Cancer Cell **(2006)** 9,425–34, http://dx.doi.org/10.1016/j.ccr.2006.04.023.
10. Le, A., Cooper, C. R., Gouw, A. M., Dinavahi, R., Maitra. A., and Dec, L.M., Proc Natl Acad Sci **(2010)** 107, 2037–42, http://dx.doi.org/10.1073/pnas.0914433107.
11. Seth, P., Grant, A., Tang, J., Vinogradov. E., Wang, X., and Lenkinski, R., Neoplasia **(2011)**13, 60–71.
12. Yoo, H. W., Suh, M. E., Park, S. W., J. Med. Chem **(1998)** 41.
13. Chu-Moyer, M. Y., Ballinger, W. E., Beebe, D. A., Berger, R., Coutcher, J. B., Day, W. W., Li, J., Mylari, B. L., Oates, P. J., Weekly, R. M. J., Med. Chem **(2002)** 45, 511, http://dx.doi.org/10.1021/jm010440g.
14. Reddy Sastry, C. V., Rao, K. S., Krishnan, V. S. H., Rastogi, K., Jain, M. L., Narayan, G. K. A. S. S., Indian J. Chem **(1990)** 29B, 396
15. Kurasawa, Y., Kim, H. S. J., Heterocycl. Chem **(1998)** 35, 1101, http://dx.doi.org/10.1002/jhet.5570350509.
16. Campiani, G., Aiello, F., Fabbrini, M., Morelli, E., Ramunno, A., Armaroli, S., Nacci, V. Garofalo, A., Greco, G., Novellino, E., Maga, G., Spadari, S., Bergamini, A., Ventura, L., Bongiovanni, B., Capozzi, M., Bolacchi, F., Marini, S., Coletta, M., Guiso, G., Caccia, S., J. Med. Chem **(2001)** 44, 305, http://dx.doi.org/10.1021/jm0010365.
17. Wroblewsk, F., La Due, J., and Proc., Soc. Exp Biol **(1955)** 90,210, http://dx.doi.org/10.3181/00379727-90-21985.
18. Avendano, C., Menendez, J. C., Medicinal Chemistry of Anticancer Drugs. Elsevier **(2008)** B 1, 1-7
19. DeVita, V. T., Chu, Jr. E., Cancer Res**. (1968)** 21, 8643-8653.
20. Tennant, D. A., Durán, R. V., Gottlieb, E., Nat Rev Cancer **(2010)** 10, 267 – 77, http://dx.doi.org/10.1038/nrc2817.
21. Vander Heiden, M. G., Nat Rev Drug Discov **(2011)** 10, 1 – 14, http://dx.doi.org/10.1038/nrd3504.
22. Neidle, S., Principles of nucleic acid structure, 5. Principles of small molecule DNA recognition, Elsevier Inc. **(2008)** pp. 132-203, http://dx.doi.org/10.1016/B978-012369507-9.50006-6.
23. Zhigang, L., Qing, Y., and Xuhong, Q., Bioorg. Med. Chem. Lett, **(2005)** 15, 3143-3146, http://dx.doi.org/10.1016/j.bmcl.2005.04.012.
24. Warburg, O., Posener, K., Negelein, E., Biochemische Zeitschrift, **(1924)** Vol. 152, pp. 319-344, (German). Reprinted in English in the book on metabolism of tumors by O. Warburg, Publisher: Constable, London, **(1930).**
25. Vazquez, A., Liu, J., Zhou, Y., Oltvai, Z., BMC systems biology **(2010)** 4, 58, http://dx.doi.org/10.1186/1752-0509-4-58.
26. Hambley, T. W., Science. **(2007)** 318(5855), 1392-1393, http://dx.doi.org/10.1126/science.1150504.
27. Chakravarty, A. R., J. Chem. Sci., **(2006)** 118, 6, 443–453, http://dx.doi.org/10.1007/BF02703941.
28. Sigman, D. S., Mazumdar, A., and Perrin, D. M., Chem. Rev, **(1993)** 93**,** 2295, http://dx.doi.org/10.1021/cr00022a011.
29. Ware, D. C., Palmer, B. D., Wilson, W. R., Denny, W. A., J. Med. Chem. **(1993)** 36, 1839-1846, http://dx.doi.org/10.1021/jm00065a006.
30. Ott, I., Gust, R., Arch. Pharm. Chem. Life Sci., **(2007)** 340, 117-126, http://dx.doi.org/10.1002/ardp.200600151.
31. Churchill, M. R., J. Amer. chem. Soc **(1969)** 91, 6518 9.
32. Orgel, L. E., J. chem. phys **(1955)** 23, 1004, http://dx.doi.org/10.1063/1.1742182.
33. Tanabe and sugano, S., J. phys. Soc. Japan **(1954)** 753,766
34. Failes, T. W., Cullinane, C., Diakos, C. I., Yamamoto, N., Lyons, J. G., Hambley, T. W., Chem. Eur. J. **(2007)** 13, 2974-2982, http://dx.doi.org/10.1002/chem.200601137.
35. Ware, D. C., Palmer, H. R., Brothers, P. J., Rickard, C. E., J. Inorg. Biochem. **(1997)** 68, 215–224, http://dx.doi.org/10.1016/S0162-0134(97)00090-1.
36. Hall, M. D., Failes, T. W., Yamamoto, N., Hambley, T. W., Dalton Trans. **(2007)** 3983-3990, http://dx.doi.org/10.1039/b707121c.