Bull. Chem. Soc. Ethiop. **2010**, 24(2), 193-199. ISSN 1011-3924
Printed in Ethiopia

SYNTHESIS, CHARACTERIZATION AND ANTI-TUMOUR ACTIVITY OF IRON(III) SCHIFF BASE COMPLEXES WITH UNSYMMETRIC TETRADENTATE LIGANDS

Fahmideh Shabani[1], Lotf Ali Saghatforoush[2] and Shahriar Ghammamy[3*]

[1]Department of Chemistry, Islamic Azad University, Young Researchers Club, Ardabil Branch, Ardabil, Iran
[2]Department of Chemistry, Khoy Payam Noor University, Khoy, Iran
[3]Department of Chemistry, Faculty of Science, Imam Khomeini International University, Ghazvin, Iran

(Received July 13, 2009; revised February 11, 2010)

ABSTRACT. The synthesis and characterization of two new iron(III) complexes, [Fe(pythsalI)]Cl_2 and [Fe(pythsalBr)]Cl_2 with the NSNO-donor tetradentate Schiff base ligands pythsalHX [(5–X-N-(2pyridylethylsulfanylethyl) salicylideneimine] (X = I, Br)) obtained from the inserted condensation of 1-(2-pyridyl)-3-thia-5-aminopentane with the respective derivative salicylaldehyde in a 1:1 molar ratio is reported. The iron(III) complexes were characterized by several techniques using elemental analysis (C, H, N), FT-IR, electronic spectra and molar conductance measurements. The elemental analysis data suggest the stoichiometry to be 1:2 [M:L] ratio formation. The molar conductance measurements reveal the presence of 1:2 electrolytic nature complexes. Infrared spectral data agreed with the coordination to the central metal ion through deprotonated phenolic oxygen, imine and pyridine type nitrogens and the thioether sulfur atoms. From ligand field spectral data an octahedral geometry is assigned to the iron(III) ion in all these complexes. These new compounds have showed anti-tumour activity against two kinds of cancer cells that are K562 (human chronic myeloid leukemia) and Jurkat (human T lymphocyte carcinoma).

KEY WORDS: Iron(III) complexes, Tetradentate ligands, Unsymmetric Schiff base, NSNO-donor, Anti-tumour, K562, Jurkat

INTRODUCTION

Transition metal macro cyclic complexes have received a great attention because of their biological activities, including anti-tumour, antibacterial, antiviral, antifungal and anticarcinogenic properties [1-5]. Such biological activities are essentially due to their ability to form tetradentate chelate with heavy metal ions, bonding through sulfur and nitrogen [6, 7]. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [8, 9].

The design, synthesis and characterization of iron complexes with Schiff base ligands play a relevant role in the coordination chemistry of iron due to their importance as synthetic models for the iron-containing enzymes [10, 12], oxidation catalysts [13, 14] and stable molecular materials based on temperature-, pressure- or light induced spin-crossover behavior [15, 16]. Schiff base ligands are considered privileged ligands, because they are easily prepared by a simple one-pot condensation of an aldehyde and primary amines in an alcohol solvent [10]. Furthermore it was found that some iron(III) complexes provide a useful structural and electronic model for the similarly coordinated iron(III) sites found in the heme iron enzymes [17]. Mascharak *et al.* [18, 19] studied the structures and properties of a number of iron(III) complexes with some amidate ligands because such complexes can be taken as a model for the metal coordination spheres of the anti-tumour drug bleomycin [20].

From a bioinorganic point of view, the iron(III) complexes with salicylidene amines ligands provide a useful structural and electronic model for the similarly coordinated iron(III) sites found in the hemi iron enzymes [21].

*Corresponding author. E-mail: shghamami@ikiu.ac.ir ; shghamamy@yahoo.com

From these points of view, it is interesting to study different types of transition metal complexes of these biologically active ligands. In this paper, the synthesis, characterization and anti-tumor properties of two iron(III) complexes have been studied.

EXPERIMENTAL

Chemical reagents

All chemicals and reagents used for the preparation of the ligands and complexes were commercial products (Merck, Germany or Fluka, Switzerland) and were used without further purification. Solvents used for reactions were purified and dried by standard procedures [22]. 5-Iodo-salcylaldehyde, 5-phenylazo-salcylaldehide, 1-(2-pyridyl)-3-thia-5-aminopentane were synthesized according to known procedures [23-25]. 2-Vinylpyridine was distilled in vacuum before using. Elemental analyses (carbon, hydrogen, and nitrogen) were determined with an Elemental CHN Analyzer Vario El III (Germany). The molar conductance of the complexes were measured in acetonitrile solution in room temperature with a Jenway 4510 conductometer instrument (Germany). Melting points were determined using an electrothermal apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectroscopic data were recorded on a Bruker spectrospin Avance (Germany) 400 MHz in $CDCl_3$ and chemical shifts are indicated in ppm relative to tetramethylsilane. The electronic spectroscopic data in 200–900 nm range were recorded in acetonitrile on a Perkin-Elmer lambda 25 spectrophotometer (USA). Infrared spectroscopic data (KBr disc, 4,000–400 cm^{-1}) were recorded on a Shimadzu FT-IR model prestige 21 spectrometer (Japan).

Cell culture

The human chronic myeloid leukemia: K562 cell line and the human T lymphocyte carcinoma: Jurkat cell line, used for treatment with the drugs, was provided. K562 and Jurkat cells were grown at 37 ^{o}C in an atmosphere containing 5% CO_2, with RPMI-1640 Medium HEPES Modification with L-glutamine and 25 mM HEPES (Sigma-Aldrich Chemie GmbH, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, USA), 2.7% sodium bicarbonate and 500 mg/L ampicillin.

Preparation and characterization of the ligands

1-(2-Pyridyl)-3-thia-5aminopentane was condensed with the respective derivative salicylaldehyde in a 1:1 molar ratio. Details are described below. The four unsymmetric tetradentate Schiff base ligands were synthesized in a similar manner [26]. A solution of 1 mmol of pyta in 5 mL absolute ethanol was added to a solution of 1 mmol of the required salicylaldehyde in 5 mL absolute ethanol to give clear yellow or light orange solutions which were gently refluxed for about 1 h. Evaporation of the solution in vacuum gave viscous liquids.

Synthesis of Schiff base ligand

The ligand Hsal-Br-pyta [5-bromo-N-(2-pyridylethylsulfanylethyl)salicylideneiminate, K^4 N,N',O,S] was synthesized by stirring 1-(2-pyridyl)-3-thia-5aminopentane (pyta) (1 mmol in 5 mL absolute ethanol) and 2-hydroxy-5-bromobenzaldehyde (1 mmol in 5 mL absolute ethanol) in reflux for 60 min, resulting in a light yellow solution containing the liquid ligand. Evaporation of the solution in vacuum gave viscous liquid. The ligand Hsal-Br-pyta was obtained as yellow micro crystals. The micro crystals were filtered off, washed with 5 mL of

cooled absolute ethanol and then recrystallized from ethanol-/chloroform (2:1, v/v). The analytical and physical data of the ligand are given below.

HsalBrpyt. Empirical formula: $C_{16}H_{17}BrN_2OS$, formula weight: 365.293 $gmol^{-1}$, yield: 83%. Color yellow, m.p. 69 °C. Analysis: C, 52.50%; H, 4.70%; N, 7.60%. Found: C, 52.60%; H, 4.69%; N, 7.66%. ^{1}H NMR (ppm): 13.20 (br s, 1H, OH), 8.55 (d, 1H, pyridinic), 8.26 (s, 1H, iminic), 7.67 (t, 1H, pyridinic), 7.39-7.21 (m, 4H, aromatic), 6.85 (d, 1H, aromatic), 3.80 (t, 2H, CH_2 aliphatic), 3.13 (t, 2H, CH_2 aliphatic), 3.00 (t, 2H, CH_2 aliphatic), 2.86 (t, 2H, CH_2 aliphatic). ^{13}C NMR (ppm): (12 C aromatic); 164.61, 160.16, 159.66, 149.32, 136.45, 134.92, 133.46, 123.25, 121.56, 119.96, 119.02, 109.95; (4 C aliphatic); 58.93, 38.36, 32.94, 31.82.

HsalIpyt. Empirical formula: $C_{16}H_{17}IN_2OS$, formula weight: 412.294 $gmol^{-1}$, yield: 71%. Color: yellow, m.p. 71 °C. Analysis: C, 46.70%; H, 4.20%; N, 6.80%. Found: C, 46.61%; H, 4.15%; N, 6.79%. ^{1}H NMR (ppm): 13.35 (br s, 1H, OH), 8.54 (d, 1H, pyridinic), 8.23 (s, 1H, iminic), 7.64 (t, 1H, pyridinic), 7.55-7.17 (m, 4H, aromatic), 6.74 (d, 1H, aromatic), 3.78 (t, 2H, CH_2 aliphatic), 3.09 (t, 2H, CH_2 aliphatic), 2.98 (t, 2H, CH_2 aliphatic), 2.84 (t, 2H, CH_2 aliphatic). ^{13}C NMR (ppm): (12 C aromatic); 164.55, 160.90, 159.67, 149.33, 140.68, 139.56, 136.52, 123.31, 121.60, 120.79, 119.57, 79.15; (4 C aliphatic); 58.93, 38.36, 32.97, 31.84.

Preparation of iron complexes

General synthesis of [Fe(pythsalX)]Cl_2 complexes. An ethanol solution of pyta (1 mmol, 5 mL) was added to ethanol solution of the required salicylaldehyde (1 mmol, 5 mL). The mixture was refluxed for 40 min, then 1 mL of metabolic NaOH was added and were continued for a further 5 min. Then an ethanol solution of $FeCl_3.6H_2O$ (1 mmol, 5 mL) was added to the ligand solution with stirring and the reaction mixture was stirred under reflux for 50 min. The resultant colored solution was left at room temperature. The product was removed by filtration, washed with cooled absolute ethanol and recrystalized from methanol or acetonitrile and dried in vacuum.

Analysis of 5-iodo-N-(2pyridylethylsulfanylethylsalcylideneiminato, K^4 N,N,O,S] iron(III) chloride dihyrate. Dark red-brown crystals, yield 68%, m.p. 274 °C dec. Anal. calcd for $C_{16}H_{20}Cl_2FeIN_2O_3S$: C, 33.47; H, 3.51; N, 4.88. Found: C, 33.30; H, 3.50; N, 4.92. FT-IR (KBr) ν 3420, 3030-3080, 2870-2910, 1617 cm^{-1}. UV (CH_3CN) λ_{max}(nm) (log ε L mol^{-1} cm^{-1}) 564 (143), 399 (4200), 325 (4550), 230 (28500). Mol. conductivity 198 µS.

Analysis of 5-bromo-N-(2pyridylethylsulfanylethylsalcylideneiminato, K^4 N,N,O,S] iron(III) chloride dihyrate. Dark brown crystals, yield 65%, m.p. 271 °C dec. Anal. calcd for $C_{16}H_{20}BrCl_2FeN_2O_3S$: C, 36.68; H, 3.77; N, 5.52. Found: C, 33.46; H, 3.82; N, 5.31. FT-IR (KBr) ν 3450, 3030-3080, 2840-2950, 1618 cm^{-1}. UV (CH_3CN) λ_{max}(nm) (log ε l mol^{-1} cm^{-1}) 565 (163), 397 (3550), 314 (3625), 238 (37150). Mol. conductivity 210 µS.

In vitro activities and cytotoxicity studies

[Fe(pythsalI)]Cl_2 and [Fe(pythsalBr)]Cl_2 complexes were assayed for cytotoxicity *in vitro* against K562 (human chronic myeloid leukemia) cells and Jurkat (human T lymphocyte carcinoma) cells.

The two cell lines were provided by the Pastour Instutiute Laboratory of Natural and Biomimetic in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [27]. Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 190 mL of cell suspension (5 x 10^4 cell/mL) were exposed to various

concentrations of complexes dissolved in sterile DMSO. After incubation periods 72 h for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were repeated six times.

The IC_{50} cytotoxicity values of the complexes were compared to those found for the starting organic bases as well as for some of the anticancer agents used nowadays, that are *cis*-platin and oxaplatin compounds [28].

The general method used for testing on anti-tumor properties of compounds was the standard testing method that has been previously described in greater detail:

After pre-incubation lasting 12 h at 37 °C in a 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration rang 0.1-200 μM for [Fe(pythsalI)]Cl_2 and 0.1-350 μM for [Fe(pythsalBr)]Cl_2 were added. The incubation lasted 72 h and at the end of this period IC_{90} and IC_{50} of the dead cells and live cells was measured by Trypan blue. IC_{90} and IC_{50} values, which are the compounds concentrations lethal for 90% and 50% of the tumour cells were determined both in control and in compounds concentrations lethal for both in compounds-treated cultures. The complexes were first dissolved in DMSO and then filtrated. The final concentration of DMSO in the growth medium was 2% (v/v) or lower concentrations without effect on cell replication [29, 30]. The corresponding 50% and 90% inhibitory doses (IC_{50} and IC_{90}) values are shown in Table 1.

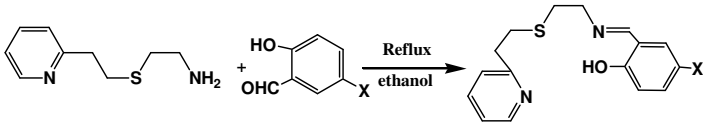
Table 1. 72 h IC_{50} and IC_{90} values (μM) obtained for two compounds.

Compound	IC_{50} for cell line		IC_{90} for cell line	
	K562	Jurkat	K562	Jurkat
[Fe(pythsalI)]Cl_2	>53	>50	>100	>99
[Fe(pythsalBr)]Cl_2	>85	>83	>175	>170

RESULTS AND DISCUSSION

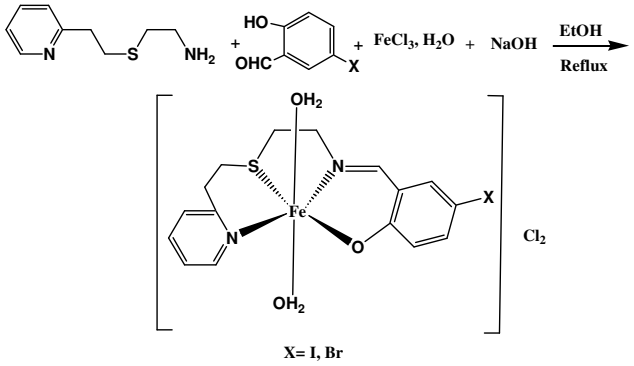
Four tetradentate monoanionic ligands pythsalHX (X = I, Br) all having an NSNO donor atom set were synthesized by the 1:1 condensation reaction of the precursor 1-(2-pyridyl)-3-thia-5-aminopentane with respective salicylaldehyde derivative in purified ethanol (Scheme 1). Iron complexation reactions of these ligands were investigated. The complexes of these ligands were obtained from a refluxing mixture of the respective ligand, methanolic NaOH and hydrated iron(III) chloride, taken in a 1:1:1 molar proportion in ethanol (Scheme 2). The Schiff base ligands and iron complexes were characterized by elemental analyses, solution electrical conductivity, FT-IR, UV-Vis and ^1H and ^{13}C NMR spectroscopy. The results are consistent with the proposed mononuclear formulation. These complexes were found to be fairly soluble in methanol, acetonitrile, dimethyl formamide and dimethyl sulfoxide and display good stability in the air at room temperature. Molar conductivities of all the four complexes are in accord with 1:2 electrolyte behaviors [31]. The structures of the ligands were confirmed by ^1H and ^{13}C NMR spectra data. In the ^1HNMR spectra of ligands, the signal for proton of –NH group was not found and it is suggested that the Schiff base ligands do not undergo keto-enol tautomerism. The FT-IR spectra of complexes compared with those of the ligands, indicate that the ν(C=N) band at 1634-1655 cm^{-1} is shifted to lower frequency by 17-28 cm^{-1} in the complexes, indicating that the ligands are coordinated to the metal ions through the nitrogen atom of the azomethine group [32]. On the other hand, the disappearance of the OH bands of the free ligands in the complexes indicates that the OH group has been deprotonated and bonded to metal ions as O^-. A relatively medium broad absorption band with maximum at 3400-3450 cm^{-1} indicates the presence of water as the elemental analyses the complexes show the presence of two moles of

water in the one mole of the complexes. The electronic spectra of the iron(III) complexes were measured in the acetonitrile solution. In general, the electronic transitions for iron(III) systems are spin forbidden and hence weak, and are often marked by charge transfer bands [33]. However, in several spin equilibrium systems, the high spin (S = 5/2) form has been characterized by transition at 555-500 nm and the low-spin (S = 1/2) form by transition at 714-625 nm [33-35]. From the spectral data of the iron(III) complexes (Figure 1) it can be seen that all of them exhibit one band at 508–568 nm which can be assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$ transition characteristic of octahedral structure [33, 36, 37]. The broad intense and poorly resolved bands between 320-450 nm may be assigned to LMCT or MLCT [34-36]. The high intensity band below 320 nm is of ligand origin assignable to intraligand n - π* / π - π* transition [36-37].



pythsalHX
X=I, Br

Scheme 1. Schematic representation of Schiff base ligands formation.



X= I, Br

Scheme 2. Schematic representation of iron(III) Schiff base complexes formation.

From the previous data: elemental analysis, molar conductance measurements, infrared and electronic spectra of the iron(III) complexes, the following can be drawn concerning the ligating property of the Schiff base as well as the stereochemistry of their corresponding complexes. The molar conductance measurements of the complexes show the 1:2 electrolytic systems. The infrared spectral data reveal one mode of complexation through nitrogen atom of azomethine and pyridine groups, oxygen atom of deprotonated phenolic group and thioether sulfur atom. The electronic spectra exhibit octahedral geometry for all of the iron(III) complexes. It is clear from the above discussion that iron(III) complexes offer a new outlook for chemotherapy. The result of anti-tumour activity show that the metal complexes exhibit anti-tumour properties and it is important to note that they show enhanced inhibitory activity compared to the parent ligand. The mechanism by which these complexes act as anti-tumour agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation [38-40].

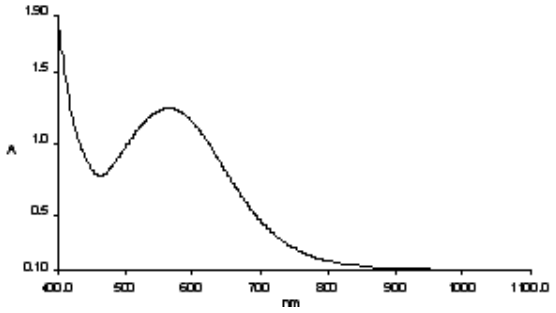


Figure 1. Electronic spectra of [Fe(pythsalI)]$Cl_2.2H_2O$.

AKNOWLEDGEMENTS

We gratefully acknowledge the financial support from the Research Council of Ardabil Islamic Azad University and many technical supports that provided by Tarbiat Modarres University.

REFERENCES

1. Liu, C.M.; Xiong, G.; You, X.Z.; Liu, Y.J.; *Polyhedron* **1996**, 15, 4565.
2. West, D.X.; Liberia, E.; Padhye, S.B.; Chikate, R.C.; Sonawane, P.B.; Kumar, A.S.; Yeranda, R.S. *Coord. Chem. Rev.* **1993**, 49, 123.
3. Canadas, M.; Torres, E.L.; Aris, A.M.; Mendrila, M.A.; Sevilla, M.T. *Polyhedron* **2000**, 19, 2059.
4. Labisbal, E.; Sousa, A.; Castineiras, A.; Graciavazquez, A., Romero, J.; West, D.X. *Polyhedron* **2000**, 19, 1255.
5. Fox, O.D.; Drew, M.G.B.; Wilkinson, E.J.S.; Beer, P.O. *Chem. Commun.* **2000**, 391.
6. Isse, A.A.; Gennaro, A.; Vianello, E. *J. Chem. Soc., Dalton Trans.* **1993**, 2091.
7. Keys, R.F.; Carter, J.J.; Englund, E.E.; Daly, M.M.; Stone, G.G.; Nilius, A.M.; Ma, Z. *J. Med. Chem.* **2003**, 46, 1795.
8. Chohan, Z.H.; Sheazi, S.K.A. *Synth. React. Inorg. Met-Org. Chem.* **1999**, 29, 105.
9. Jayabalakrishnan, C.; Natarajan, K. *Synt. React. Inorg. Met-Org. Chem.* **2001**, 31, 983.
10. Kannappan, R.; Tanasae, S.; Mutikainen, I.; Turpeinen, U.; Reedijk, J. *Polyhedron* **2006**, 25, 1646.
11. Sivasubramanian, V.K.; Ganesan, M.; Rajagopal, S.; Ramaraj, R. *J. Org. Chem.* **2002**, 67, 1506.
12. Fujii, H.; Kurahashi, T.; Ogura, T. *J. Inorg. Biochem*. **2003**, 96, 133.
13. Bedford, R.B.; Bruce, D.W.; Frost, R.M.; Goodby, J.W.; Hird, M. *Chem. Commun.* **2004**, 18, 2822.
14. Katsuki, T. *Chem. Soc. Rev.* **2004**, 33, 437.
15. Bhadbhade, M.M.; Srinivas, D. *Polyhedron* **1998**, 17, 2699.
16. Brewer, C.T.; Brewer, G.; Jameson, G.B.; Kamaras, P.; May, L.; Rapta, M. *J. Chem. Soc. Dalton Trans.* **1995**, 37.
17. Zhu, S.; Brennessel, W.W.; Harrison, R.G.; Que Jr., L. *Inorg. Chim. Acta* **2002**, 337, 32.
18. Rowland, J.M.; Olmstead, M.M.; Mascharak, P.K. *Inorg. Chem.* **2001**, 40, 2810.
19. Noveron, J.C.; Olmstead, M.M.; Mascharak, P.K. *J. Am. Chem. Soc.* **2001**,123, 3247.

20. Burger, R.M. *Struct. Bond.* **2000**, 97, 287.
21. Canali, L.; Sherrington, D.C. *Chem. Soc. Rev.* **1999**, 28, 85.
22. Perrin, D.D.; Armarego, W.L.F. *Purification of Laboratory Chemicals*, 3rd ed., Pergamon: Oxford; **1980**; pp 68, 174, 217.
23. Pavia, R.M.; Cohen, P.M.; Dilley, J.C.; Dubuc, R.G.; Duginal, L.T.; Forman, W.F.; Hediger, E.M.; Milota, G.; Powers, S.T.; Sucholeiki, I.; Zhou, S.; Hangauer, G.D. *Bioorg. Med. Chem.* **1996**, 4, 659.
24. Khandar, A.A.; Rezvani, Z. *Polyhedron* **1998**, 18, 129.
25. Kaasjager, V.E.; Puglisi, L.; Bouwman, E.; Driessen, W.L.; Reedijk, J. *Inorg. Chim. Acta* **2000**, 310, 183.
26. Daneshvar, N.; Entezami, A.A.; Khandar, A.A.; Saghatforoush, L.A. *Polyhedron* **2003**, 22, 1437.
27. Zhao, G.; Lin, H.; Zhu, S.; Sun, H.; Chen, Y.; *J. Inorg. Biochem.* **1998**, 70, 219.
28. Kim, Y.S.; Song, R.H.; Chung, C.; Jun, M.J.; Sohn, Y.S. *J. Inorg. Biochem.* **2004**, 98, 98.
29. Ishida, J.; Wang, H.K.; Bastow, K.F.; Hu, C.Q.; Lee, K.H. *Bioorg. Med. Chem. Lett.* **1999**, 9, 3319.
30. Son, J.K.; Zhao, L.X.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T.C.; Jeong, B.S.; Lee, C.S.; Lee, E.S. *Europ. J. Med. Chem.* **2007**, 42, 1.
31. Szafran, Z.; Pike, R.M.; Singh, M.M. *Microscale Inorganic Chemistry*, Wiley: New York; **1991**, p 104.
32. Williams, D.H.; Fleming, I. *Spectroscopic Methods in Organic Chemistry*, 4th ed., McGraw Hill: London; **1989**; pp 52-54, 73, 135.
33. Sarkar, S.; Dey, K. *Spectrochim. Acta Part A* **2005**, 62, 383.
34. Maeda, Y.; Tsutsumi, N.; Yakashima, Y. *Inorg. Chem.* **1984**, 23, 2440.
35. Gaber, M.; Issa, R.M.; Ghoniem, M.M.; El-Baradie, K.Y. *Egypt. J. Chem.* **1991**, 34, 107.
36. Rahman, Sk.H.; Chowdhury, H.; Bose, D.; Ghosh, R.; Hung, C.H.; Ghosh, B.K.; *Polyhedron* **2005**, 24, 1755.
37. Madha, N.T.; Radhakrishnan, P.K.; Grunert, M.; Weinberger, P.; Linert, W. *Thermochim. Acta* **2003**, 407, 73.
38. Shabani, F.; Ghammamy, Sh.; Mehrani, Kh. *Bioinorg. Chem. Applications* **2008**, doi: 10.1155/2008/501021.
39. Ghammamy, Sh.; Shabani, F. *Der Pharma Chemica* **2009**, 1, 30.
40. Ghammamy, Sh.; Shabani, F. *Der Pharma Chemica* **2009**, 1, 124.