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Original article

New diketone based vanadium complexes as insulin mimetics

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Abstract

Since 1985, when Heyliger et al. first reported the in vivo insulin mimetic activity of oral vanadate, extensive studies exploring vanadium chemistry, including the synthesis of novel complexes and their biological effects both in vitro and in vivo have been pursued. Such complexes have emerged as possible potential agents for diabetes therapy. Among the several existing compounds, diketone based vanadium complexes have been chosen for the current study. Two new complexes namely bisdimethylmalonatooxovanadium(IV) and bisdiethylmalonatooxovanadium(IV) have been synthesized and characterized by UV–visible, FTIR and mass spectral studies. The antidiabetic activity of the complexes was proved by animal study. The results show that the above complexes have comparable antidiabetic potential with respect to the standard drug as well as with bisacetylacetonatooxovanadium(IV) which has been studied earlier by Reul et al.

Keywords: Oxovanadium (IV); Insulin mimetics; Dimethylmalonate; Diethylmalonate

1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease resulting from insulin deficiency or insulin resistance. DM is threatening because of the development of many severe secondary complications like atherosclerosis, renal dysfunction, liver toxicity, cardiac abnormalities and diabetic retinopathy. Generally DM is classified as Type I Insulin Dependent Diabetes Mellitus (IDDM) caused by low or insufficient secretion of insulin by pancreas and Type II Non-Insulin Dependent Diabetes Mellitus (NIDDM) caused due to insufficient utilization of insulin [1, 2]. Type I can fairly be controlled by daily subcutaneous injections of insulin which causes pain and stress. Type II DM is treated by several types of synthetic therapeutics [3]. Orally active therapeutic agents in the place of painful insulin injection for Type I DM and synthetic drugs without side effects for Type II DM have become an issue of major concern. Vanadium complexes are one such class of compounds studied for this purpose.

In biological system, vanadium is found predominantly as vanadate (+5) and vanadyl (+4) forms. The very first report of vanadium salts being used as a metallotherapeutic agent appeared in 1899. After that in 1985, Heyliger et al. [4] first reported the in vivo insulin mimetic activity of oral vanadate. Further studies proved that vanadium compounds are effective for lowering plasma glucose levels in diabetic animals [5–10].

Very recently, in 2003, the first phase I clinical trial of vanadium based pharmaceutical agent bis(ethylmaltolato) oxovanadium(IV) (BEOV), KP-102 was completed by Medieval Ltd., Manchester, UK without any adverse effects [11]. It has been proved that vanadium treatment results in the correction of several diabetes related abnormalities, in carbohydrate and lipid metabolism. The preliminary results showed that the complexes of vanadium with organic ligands seemed to be safer than inorganic vanadium salts [12]. In order to achieve better absorption and to minimize the dosage level, it is appropriate to administer vanadium in the form of organic matrix.

Over the past 25 years, many pharmacologically interesting ligands have been complexed with vanadium and are said to possess synergistic effect as effective antidiabetic agents. To mention a few are 3-methyl, 2-hydroxypyrone (maltol),

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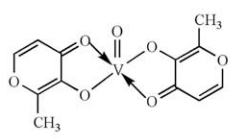
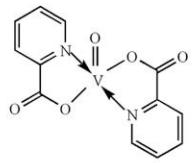
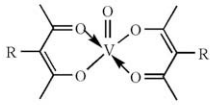
pyridine 2-carboxylic acid (picolinic acid). pentanedione (acetylacetone) etc., and their insulin mimetic potential have been assessed mainly by Sakurai, Chris Orvig and McNeill groups [13–19]. The structures of the complexes are shown below:



Bis maltolato oxovanadium (IV)



Bis picolinato oxovanadium (IV)



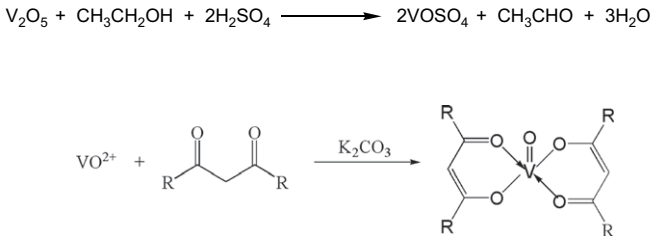
Bis acetylacetonato oxovanadium (IV)

We have chosen diketone based vanadium complexes for the current study based on the findings that bisacetylacetonatooxovanadium(IV) has already been reported for its insulin mimetic activity by Reul et al. [18] and others [19–21]. Bis (acetylacetonato)oxovanadium(IV), $[VO(acac)_2]$ was first prepared by Rowe and Jones in 1957 [22]. A variety of derivatives has been prepared and synthesized in which the terminal methyl groups in the acetylacetone ligand were substituted [23,24]. Different pathways have been proposed to understand the mechanism of action of vanadium complexes in treating diabetes. It may exert its effects through inhibition of protein tyrosine phosphatases [25,26]. Recently it has been proposed that the insulin mimetic properties of vanadium may be attributed to the inhibition of key enzyme phosphodiesterase which is involved in carbohydrate metabolism [27].

2. Results and discussions

2.1. Preparation of the complex

Scheme 1 shows the reactions of the ligands, namely, acetylacetone, dimethylmalonate (DMM) and diethylmalonate (DEM) with vanadyl sulphate from which the corresponding oxovanadium(IV) complexes were prepared.



Scheme 1.

2.2. Characterization techniques

The above complexes were characterized by FTIR, UV–visible and mass spectroscopic techniques.

2.2.1. UV–visible spectra

All three complexes exhibit four transitions in UV and visible regions in DMSO. All peaks observed in the visible region are metal based d–d transitions and the one in the UV region is due to $\pi-\pi^*$ transition of the ligands (Refer Table 1).

2.2.2. FTIR

The IR spectra of the ligands and their complexes are compared. There is a shift in the carbonyl frequency in all the three complexes showing their involvement in binding with the vanadium metal. The characteristic oxovanadium stretching frequency is also observed. (Refer Table 2).

2.2.3. Mass spectra

Mass spectral analysis was performed in solid state using direct inlet method by taking EI mass spectra. All the complexes exhibit their parent ion peaks along with characteristic fragmentation patterns.

2.3. Evaluation of antidiabetic activity

To assess the antidiabetic activity of the vanadium complexes, namely, $[VO(DMM)_2]$ and $[VO(DEM)_2]$, animal study was carried out. The glucose level, protein level, total cholesterol level along with the body weight of the animal are monitored before and after treatment with the drug. The reported compound $[VO(acac)_2]$ was also subjected to the study for comparison and the following analyses were carried out.

2.3.1. Estimation of glucose

In Fig. 1, glucose levels before and after treatments of the drugs are shown. There is a considerable decrease in the glucose level after treatment with the drug compounds. It is more effective with respect to $[VO(DEM)_2]$ than $[VO(DMM)_2]$. The values are comparable with that of the reported compound $[VO(acac)_2]$ and also with the standard antidiabetic drug glibenclamide.

2.3.2. Estimation of protein

Under DM conditions, the protein level may decrease and hence we have also studied the protein profile. In Fig. 2, the protein levels for each test group before and after treatment are shown. We observe only marginal increase in the protein levels after treatment and is more prominent with respect to $[VO(DEM)_2]$. The comparison is also made with $[VO(acac)_2]$ and a standard drug. Studies on the estimation of proteins before and after treatment are subject to discussion. However,

Table 1
UV–visible absorption bands shown by the ligands and their vanadium complexes

Ligand	λ_{max} (nm)	Complexes	$\pi-\pi^*$ (nm) (ε: $M^{-1}\,cm^{-1}$)	$d_{xy}-d_{z^2}$ (nm) (ε: $M^{-1}\,cm^{-1}$)	$d_{xy}-d_{x^2-y^2}$ (nm) (ε: $M^{-1}\,cm^{-1}$)	$d_{xy}-d_{xz}, d_{yz}$ (nm) (ε: $M^{-1}\,cm^{-1}$)
Acetylacetone ($CHCl_3$)	248	$[VO(acac)_2]$	308 (6000)	402 (15,500)	597 (45)	680 (52)
Dimethylmalonate (DMSO)	318	$[VO(DMM)_2]$	294 (16,600)	324 (21,800)	526 (20)	606 (50)
Diethylmalonate (DMSO)	245	$[VO(DEM)_2]$	292 (16,400)	567 (12,000)	611 (40)	889 (20)

a detailed study [28] indicates that there is a net protein loss during insulin deprivation.

2.3.3. Estimation of total cholesterol

As diabetes is always associated with atherosclerosis, the effect of the drugs on total cholesterol level before and after treatment with the drug are considered. From Fig. 3, it is shown that the decrease in the total cholesterol level is also significant after treatment with the drug compounds. When compared with the alloxan treated group, the two synthesized compounds have shown considerable reduction in the total cholesterol level.

2.3.4. Body weight

In Fig. 4, the body weight of each group of the animals before and after treatment with the drug is shown. The body weight profile indicates that there is no negative effect on body weight of the animal after treatment with both $[VO(DMM)_2]$ and $[VO(DEM)_2]$. These complexes show comparable results with that of $[VO(acac)_2]$ and the standard drug.

2.3.5. Diketone based vanadium complexes

β-Diketones and related derivatives are considered as a class of very important ligands in the field of coordination chemistry. Their complexes with many metal ions have been extensively studied. Due to the presence of two oxygen donor atoms and facile keto–enol tautomerism. These complexes easily coordinate with metal ions after deprotonating the enolic hydrogen atom and provide stable metal complexes with six membered chelate rings [29]. $[VO(acac)_2]$ exhibits a square pyramidal geometry and has shown promising insulin mimetic properties. This led to synthesis of complexes with derivatives of β-diketonato ligand system with systematic variation of substituents which include both symmetrical or asymmetrical modification of the parent ligand leading to the formation of corresponding $[VO(acac)_2]$ like complexes [30,31].

Mahroof-Tahir et al. had synthesized and characterized vanadium complexes of β-diketonato ligand system with systematic variations of electronic and steric factors. Accordingly, two complexes $[VO(tmh)_2]$ (tmh = 2,2,6,6-tetramethyl-3,5-heptanedione) and $[VO(hd)_2]$ (hd = 3,5-heptanedione) had been synthesized and characterized by different spectroscopic techniques. Elemental and mass spectral analysis support the presence of two β-diketonato ligands per VO^{2+} unit [32]. Based on the above literature survey, it is appropriate that both the new complexes $[VO(DMM)_2]$ and $[VO(DEM)_2]$ can be considered as β-diketonate based vanadium complexes whose insulin mimetic activity is comparable to similar complexes.

Shuang-Qing Zhang et al. had evaluated the pharmacodynamics and pharmacokinetics study of $[VO(acac)_2]$ in rats. Pharmacokinetics study was carried out using non-diabetic and diabetic rats by subcutaneous and intragastric administrations at single dose or multiple doses. $[VO(acac)_2]$ resulted in a significant decrease of plasma glucose levels in diabetic rats [33]. Based on the above study, our compounds $[VO(DMM)_2]$ and $[VO(DEM)_2]$ are expected to also possess antidiabetic activity. These complexes indeed show activity comparable to $[VO(acac)_2]$ and the standard drug glibenclamide. In the animal study, glucose level, total cholesterol levels, protein levels are also estimated before and after treatment of the animal groups (test groups) with the drugs which are also very favorable to the new compounds as insulin mimetics.

3. Conclusion

Two new complexes namely, bisdimethylmalonatooxovanadium(IV) and bisdiethylmalonatooxovanadium(IV) have been synthesized, characterized and tested for their insulin mimetic activity. These complexes showed comparable results to that of standard antidiabetic drug glibenclamide which may be of significance to the study of antidiabetic vanadium complexes. However, effect of dose variation to reduce toxicity and chronic testing need to be done to improve its efficacy as antidiabetic agent and it is underway.

4. Experimental

4.1. Materials and methods

V_2O_5 (S.D. Fine), acetylacetone (Nice chemicals), diethylmalonate (CDH), dimethylmalonate (Sisco Research Lab), conc. H_2SO_4, ethanol, DMSO (S.D. Fine), potassium

Table 2
IR stretching frequencies of the ligands and their vanadium complexes

Ligand	Stretching frequency (cm^{-1})	Complex	Stretching frequency (cm^{-1})
Acetylacetone	C=O: 1726, 1706; V=O: nil	$[VO(acac)_2]$	C=O: 1557; V=O: 998
Dimethylmalonate	C=O: 1736 (doublet); V=O: nil	$[VO(DMM)_2]$	C=O: 1622; V=O: 975
Diethylmalonate	C=O: 1735 (doublet); V=O: nil	$[VO(DEM)_2]$	C=O: 1622; V=O: 983

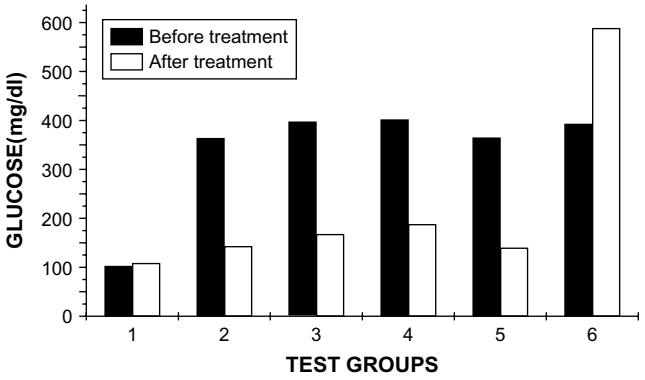


Fig. 1. Glucose lowering before and after treatment (Refer Table 3 for different test groups).

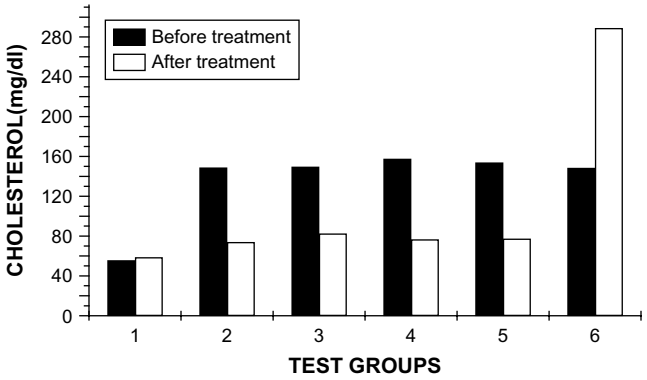


Fig. 3. Total cholesterol levels before and after treatment (Refer Table 3 for different test groups).

carbonate (Qualigens), standard rodent pellet diet (Hindustan Lever & Co., Bangalore), alloxan monohydrate (Sigma Aldrich). All the chemicals and the solvents were of analytical grade (99.9% purity).

4.2. Synthesis

The complexes were synthesized in two steps: in the first step, vanadium pentoxide was reduced to vanadyl sulphate with conc. H_2SO_4 and ethanol. In the second step, it was mixed with the ligand (acetylacetone, dimethylmalonate or diethylmalonate) in 1:2 molar ratio and was neutralized by adding K_2CO_3 with stirring. The product was filtered, washed with water, ethanol and dried in air.

The structures of the complexes are tentatively assigned to be square pyramidal with oxoligand at the apex position. This assignment is based upon the similarities in spectroscopic and other properties of these complexes with previously reported complexes with diketonato ligands.

4.3. Characterization

The electronic spectra were recorded on a Shimadzu-1601 double beam UV–visible spectrometer, FTIR on Thermo Nicolet, Avatar 330 FTIR spectrometer, USA, EI mass on Shimadzu QP 50000 spectrometer. The analyzer grade kit for animal study was obtained from Erba Diagnostics, Germany.

4.4. Glucose-lowering studies

4.4.1. Test groups

The animal study was carried out according to the guidelines of Animals Ethics Committee using adult wistar albino rats (150–200 g) of either sex. The rats were maintained in standard polypropylene cages at room temperature of $\pm 25\,^{\circ}C$ and were divided into four groups: normal control (6 rats), diabetic control - glibenclamide (6 rats), drug treated diabetics (6 rats in each group) and diabetic control – alloxan monohydrate (6 rats).

4.4.2. Experimental protocol

The acclimatized animals were kept fasting for 24 h with water ad libitum and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After 1 h, the animals were provided feed ad libitum. The blood glucose levels were checked before and 48 h after alloxanisation by withdrawing blood from retro-orbital puncture under mild ether anesthesia. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This was observed at the end of alloxanisation. The

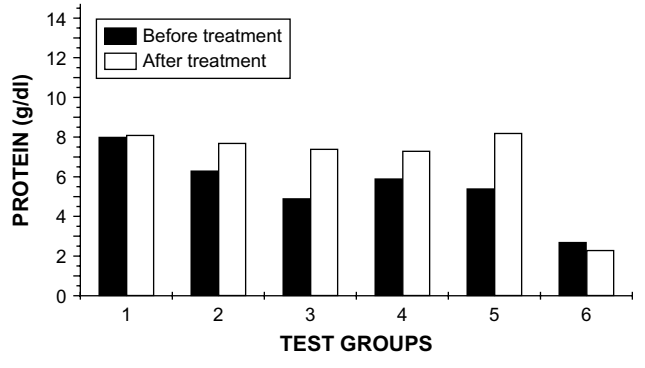


Fig. 2. Protein levels before and after treatment (Refer Table 3 for different test groups).

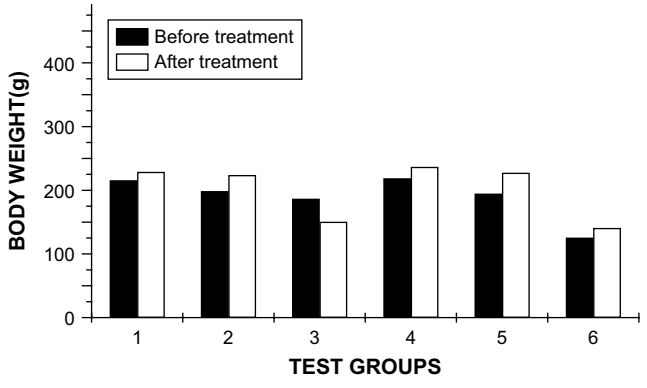


Fig. 4. Body weight before and after treatment (Refer Table 3 for different test groups).

Table 3
Test groups and their dosage levels

Groups	Test groups	Dosage levels (kg^{-1})
1	Normal control (only vehicle)	2 ml
2	Diabetic control – treated (glibenclamide)	2.5 mg
3	Drug treated-1 [$VO(acac)_2$]	25 mg
4	Drug treated 2 [$VO(DMM)_2$]	25 mg
5	Drug treated 3 [$VO(DEM)_2$]	25 mg
6	Diabetic control – untreated (alloxan monohydrate)	120 mg

animals were segregated into 6 groups of 6 rats each, say, normal control (only vehicle), diabetic control (glibenclamide), drug treated 1,2,3 [$VO(acac)_2$, $VO(DMM)_2$, $VO(DEM)_2$, respectively] and diabetic control – untreated (alloxan monohydrate). Table 3 shows the classification of test groups of 6 rats each and their dosage levels.

4.4.3. Treatment procedure

Treatment was initiated 48 h after alloxanisation. The synthesized compounds [$VO(acac)_2$, $VO(DMM)_2$, $VO(DEM)_2$] were given orally for a period of one week at a dosage level of 25 mg/kg (about 5 mg, 4 mg, 3 mg V/kg, respectively) everyday. The dosage was arrived from the reported study of similar complexes. At the end of one week, the blood samples were collected from each rat of individual groups under mild ether anesthesia and all the analyses were carried out. The collected blood samples were centrifuged at 2000 rpm for 10 min and supernatant serum was separated from each blood sample into respective tubes by suitable micropipettes. The separated serum was taken for analyses of glucose, triglycerides and protein levels. The body weight of the animals before and after treatment was also observed throughout the study.

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References

[1] H. Sakurai, Chem. Rec. 2 (2002) 237–248.
[2] WHO, Diabetes Mellitus, Report of a WHO Study Group, WHO Technical Report Series, vol. 727, 1085, pp. 91–113.
[3] D.A. Greene, A.A. Sima, M.J. Stevens, E.L. Feldman, P.D. Killen, D.N. Henry, T. Thomas, J. Dananberg, S.A. Lattimer, Diabetes Metab, Rev. 9 (1993) 189–217.
[4] C.E. Heyliger, A.G. Tahiliani, J.H. McNeill, Science 227 (1985) 1474–1477.
[5] K.H. Thompson, J. Chiles, V.G. Yuen, J. Tse, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 98 (2004) 683–690.
[6] R.A. Pederson, S. Ramanadham, A.M.J. Buchan, J.H. McNeill, Diabetes 38 (1989) 1390–1395.
[7] S. Dai, K.H. Thompson, J.H. McNeill, Pharmacol. Toxicol. 74 (1994) 101–109.
[8] A.B. Goldfine, D.C. Simonson, F. Folli, M.E. Patti, C.R. Kahn, J. Clin. Endocrinol. Metab. 80 (1995) 3311–3320.
[9] G. Boden, X. Chen, J. Ruiz, G.D. van Rossum, S. Turco, Metabolism 45 (1996) 1130–1135.
[10] M. Halberstam, N. Cohen, P. Shlimovich, L. Rossetti, H. Shamoon, Diabetes 45 (1996) 659–666.
[11] C. Orvig, K.H. Thompson, B.D. Liboiron, J.H. McNeill, V.G. Yuen, J. Inorg. Biochem. 96 (2003) 14 (Abstr.) (Presented by C. Orvig at ICBIC 11, Cairns, QLD, Australia, 2003.).
[12] D.C. Crans, J.J. Smee, E. Gaidamauskas, L. Yang, Chem. Rev. 104 (2004) 849–902.
[13] P. Poucheret, S. Verma, M.D. Grynpas, J.H. McNeill, Mol. Cell. Biochem. 188 (1998) 73–80.
[14] H. Sakurai, K. Tsuchiya, M. Nukatsuka, M. Sofue, J. Kawada, J. Endocrinol. 126 (1990) 451–459.
[15] J.H. McNeill, V.G. Yuen, H.R. Hoveyda, C. Orvig, J. Med. Chem. 35 (1992) 1489–1491.
[16] V.G. Yuen, C. Orvig, K.H. Thompson, J.H. McNeill, Can. J. Physiol. Pharmacol. 71 (1993) 263–269.
[17] H. Sakurai, H. Yasui, J. Trace Elem. Exptl. Med. 16 (2003) 269–280.
[18] B.A. Reul, S.S. Amin, J.P. Buchet, L.N. Ongemba, D.C. Crans, S.M. Brichard, Br. J. Pharmacol. 126 (1999) 467–477.
[19] K.H. Thompson, J.H. McNeill, C. Orvig, Chem. Rev. 99 (1999) 2561–2572.
[20] K.H. Thompson, C. Orvig, Coord. Chem. Rev. 219–221 (2001) 1033–1053.
[21] D.C. Crans, J. Inorg. Biochem. 80 (2000) 123–131.
[22] R.A. Rowe, M.M. Jones, Inorg. Synth. 5 (1957) 114–115.
[23] N.S. Al-Niaimi, A.R. Al-Karaghouli, S.M. Aliwi, M.G. Jalhoom, J. Inorg. Nucl. Chem. 36 (1974) 283–288.
[24] B.J. Pandya, P.K. Bhattacharya, Indian J. Chem. 25A (1986) 776–778.
[25] G. Swarup, S. Cohen, D.L. Garbers, J. Biol. Chem. 256 (1981) 8197–8201.
[26] A.S. Tracey, M.J. Gresser, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 609–613.
[27] H. Sakurai, Y. Kojima, Y. Yoshikawa, K. Kawabe, H. Yasui, Coord. Chem. Rev. 226 (2002) 187–198.
[28] Michael Charlton, Sreekumaran Nair, J. Nutr. 128 (1998) 323S–327S.
[29] Mannar R. Maurya, Coord. Chem. Rev. 237 (2003) 163–181.
[30] G.T. Morgan, H.W. Moss, J. Chem. Soc. 103 (1914) 78–90.
[31] P.K. Hon, R.L. Belford, C.E. Pfluger, J. Chem. Phys. 43 (1965) 1323–1333.
[32] M. Mahroof-Tahir, Dan Brezina, Naheed Faatima, Muhammad Iqbal Choudhary, Atta-ur-Rahman, J. Inorg. Biochem. 99 (2005) 589–599.
[33] S.-Q. Zhang, Xu-Ying Zhong, Wan-Liang Lu, Li Zheng, Xuan Zhang, Feng Sun, Gui-Ying Fu, Qiang Zhang, J. Inorg. Biochem. 99 (2005) 1064–1075.