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Research paper

Synthesis, structure, chemical and bioactivity behavior of eight chromium(III) picolinate derivatives Cr(R-pic)$_3$

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ABSTRACT

The worldwide use of chromium(III) picolinate Cr(pic)$_3$ as nutrition additives has aroused more and more controversies. To reevaluate the safety and validity of Cr(pic)$_3$, seven new derivatives Cr(R-pic)$_3$ (pic = picolinic acid, R = H (**1**), 5-Br (**2**), 5-CF_3 (**3**), 4-Cl (**4**), 5-COOH (**5**), 3-CH_3 (**6**), 5-OH (**7**), 3-OH (**8**)) were synthesized and characterized by X-ray crystal diffraction, ESI-MS, IR and elemental analysis. It was found that different substituent group affected physicochemical activities of the complex such as the Fenton-like reaction and oxidation reaction. Especially, –OH group derivatives lose their hydroxyl radical-generation and Cr(VI)-generation abilities comparing with halogen group in tube experiment. Even so, these differences in chemistry properties may be ignored in live cells and animal tests: no obvious cellular damage (MTT assay) and tissue injury (acute toxicity study) were observed for both Cr(pic)$_3$ and its derivatives. In addition, hypoglycemic activity study indicated that these Cr(III) complexes have no significant influence than $CrCl_3$ salt on the blood glucose, serum insulin, total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein of diabetic mice through two months' study. Therefore, these substituent group is unable to improve the biological activities of Cr(pic)$_3$ obviously and the validity of Cr(pic)$_3$ used as a nutrition additives is doubted.

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1. Introduction

Cr(III) was proposed to be an essential element for its efficiency in carbohydrate and lipid metabolism in the 1950s [1], and it has been suggested to be an artificial second messenger to amplify insulin signal [2]. As a man-made complex, Cr(pic)$_3$ has become one of the best-selling food additives [3], and a plenty of investigations about it have been made to better understand the role of Cr (III) in anti-diabetes. Till now, the validity and safety of Cr(pic)$_3$ remains disputable [4] for two reasons: (1) Cr(pic)$_3$ cannot be biologically active form of Cr(III) for the complex possessing no inherent biological activity itself [5], and (2) previous studies have revealed that Cr(pic)$_3$ can generate hydroxyl radical (·OH) in the presence of H_2O_2/vitamin C to cleavage DNA [6]. Thus the status of Cr(III) as an essential element has recently been challenged, and the essentiality of Cr(III) is a matter of current debate [7].

Despite no acute toxicity was observed *in vivo* through two years' animal experiments [8], Cr(pic)$_3$ was supposed to be unsafe. There is increasing evidence that the risk of Cr(pic)$_3$ derives from the ligands for picolinate is mildly clastogenic by itself [6]. Hence, picolinate is always used as ligands for anticancer complexes and exhibits well effect [9,10]. To improve the biological activity and lower the toxicity of Cr(III) complexes, varieties of new complexes such as [Cr(D-Phe)$_3$] [11], NBC [12], CDNC [13,14], LMWCr [15], [Cr(met)$_3^+$] [16,17] and CrSA [18] have been synthesized. In addition, the toxicity and hypoglycemic activity of these Cr(III) complexes are evaluated by animal study. We previously reported a direct relation between ·OH generation and the physicochemical properties of Cr(pic)$_3$ derivatives by synthesis and research of three new Cr(pic)$_3$ derivatives, Cr(6-CH_3-pic)$_3$, Cr(3-NH_2-pic)$_3$, and [Cr(6NH_2-pic)$_2$(H_2O)$_2$]·NO_3 [19]. However, systematic studies on the safety and activity of Cr(pic)$_3$ and its derivatives are still needed.

The present study provides seven new chromium(III) picolinate derivatives Cr(R-pic)$_3$ (pic = picolinic acid, R = H (**1**), 5-Br (**2**), 5-CF_3 (**3**), 4-Cl (**4**), 5-COOH (**5**), 3-CH_3 (**6**), 5-OH (**7**), 3-OH (**8**)), physicochemical properties and biological activity of these complexes. Firstly, ·OH and Cr(VI) generation of Cr(pic)$_3$ was measured by Fenton-like reaction [20] and H_2O_2 oxidation respectively *in vitro* in neutral aqueous media (pH 7.4) [21]. Then, cell damage, acute toxicity and hypoglycemic activity of Cr(pic)$_3$ derivatives were carried out. Herein, we are trying to improve the safety and hypoglycemic activity of Cr(pic)$_3$ by the substituent effect, and the results of our investigations will try to reevaluate the use of chromium picolinate complexes as food additives.

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2. Experimental

2.1. Materials and chemicals

$Cr(NO_3)_3{\cdot}9H_2O$, $CrCl_3{\cdot}6H_2O$, H_2O_2, ascorbic acid, 2-thiobarbituric acid, phosphate and all the R-pic ligands including pyridine-2-carboxylic acid (pic, **L1**), 5-bromine-2-carboxylic acid (5-Br-pic, **L2**), 5-(trifluoromethyl)pyridine-2-carboxylic acid (5-CF_3-pic, **L3**), 4-chloropyridine-2-carboxylic acid (4-Cl-pic, **L4**), 2,5-pyridine-2-carboxylic acid (5-COOH-pic, **L5**), 3-methylpyridine-2-carboxylic acid (3-CH_3-pic, **L6**), 5-hydroxypicolinic acid (5-OH-pic, **L7**), 3-hydroxy-2-pyridinecarboxylic acid (3-OH-pic, **L8**) were obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Dulbecco minimum essential media (DMEM), RPMI medium 1640, trypsin digestive juices, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and fetal bovine serum were from Solarbio Science & Technology Co. LTD (Beijing, China). Unless other indicated, all the reagents were used without purification.

Crystallographic data of Cr(III) complexes were collected on a D8 Venture X-ray diffractometer (Bruker, Germany). ESI-MS spectra were recorded on an Agilent 6520 Accurate-Mass QTOF LC/MS mass spectrometer in DMSO. UV–visible (UV–vis) spectra and elemental analyses were estimated on a Varian 50 Bio spectrophotometer and a Vario EL III analyzer respectively. IR spectra were measured with a Bruker TENSOR 21 FT-IR spectrophotometer. Water-jacketed CO_2 cell incubator was from Shanghai Lishen Scientific Instruments Co., Ltd. The Animal center of First Hospital of Shanxi Medical University (Shanxi Province, China) approved the animal experimental protocols with the qualified number SCXK (JIN) 2015–0001.

2.2. Preparation of Cr(R-pic)$_3$

$Cr(pic)_3{\cdot}(H_2O)$ (**1**) were prepared according to the literature [22]. $Cr(R\text{-}pic)_3$ complexes **2–8** were obtained by the similar methods (Scheme 1). A solution of $Cr(NO_3)_3{\cdot}9H_2O$ (0.20 g, 0.50 mmol) was added to the mixed solution of R-pic ligands (1.5 mmol) and NaOH (1.5 mmol, 20 ml) dropwise and refluxed for 1 h. Pink crystals suitable for X-ray single-crystal diffraction were obtained after allowing the filtrate of the mixture stand for 1 week at room temperature. These complexes were characterized by IR, ESI-MS, elemental analysis, and/or X-ray single-crystal diffraction (XRD) (Figs. S1–S4). The detailed synthesis process and structure characterization of complexes **1–8** by IR, ESI-MS and elemental analysis are shown in the Supporting information (Figs. S5–S12 and Figs. S21–S25 for IR and ESI-MS respectively).

2.3. X-ray diffraction

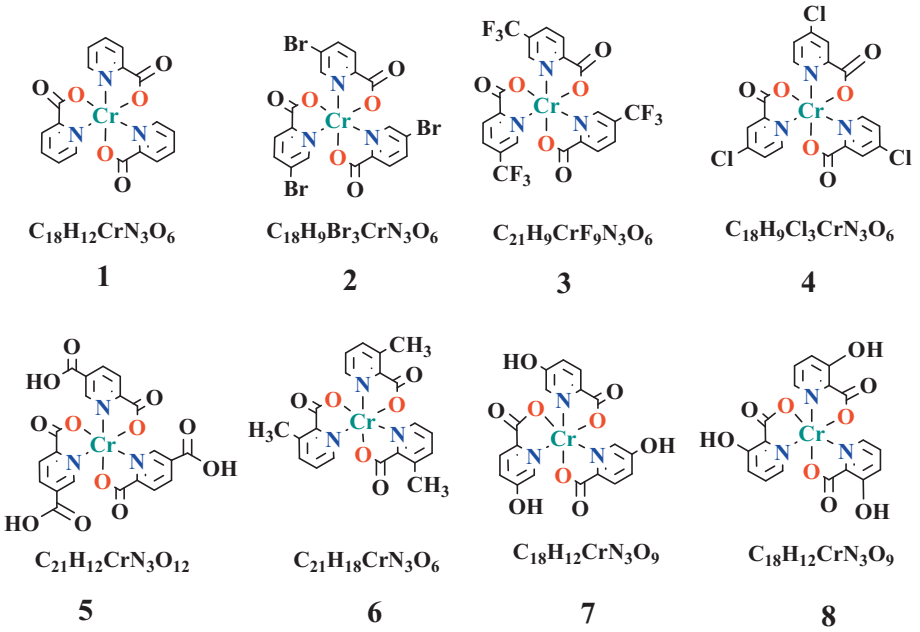
The XRD measurements for complexes **1**, **2**, **4**, and **6** were carried out with a CCD detector equipped with graphite-monochrome Mo Kα radiation. The structures of all complexes mentioned above were obtained by direct methods with SHELXL-97 and refined by full-matrix least-squares techniques on F^2 with SHELXL-97 [23]. The ORTEP diagram of complexes **1**, **2**, **4** and **6** were demonstrated in Fig. 1, and the crystallographic data, structure refinements, bond lengths and angles were listed in Table 1 and Table 2 (CCDC numbers for complexes **2**, **4**, and **6**: 1500350, 1500280 and 1500281).

2.4. Fenton-like reaction

Hydrogen peroxide (1.0×10^{-4} M) was added to the mix solution of medium (PBS, 1640 or HSA)/Fe(EDTA)/complexes **1–8** (1.0×10^{-4} M, PBS), D-2-Deoxyribose (4.0×10^{-3} M) and ascorbic acid (1.0×10^{-4} M) in PBS/RMPI medium 1640/HSA (1×10^{-4} M, PBS). After 3 h of water bath (37 °C), 2-thiobarbituric acid (2.8% w/V, 300 μL) and trichloroacetic acid (1% w/V, 5 mL) were added to the reaction mixture (2 mL) consecutively. The final solution turned pink from colorless with a maximum absorbance at 532 nm after 30 min of water bath (90 °C) [20]. In this experiment, Fe(EDTA) act as control group and medium only as blank.

2.5. Oxidation reaction

With the addition of complexes **1–8** (1×10^{-4} M, PBS, pH 7.4) to the solution (PBS/RPMI medium 1640/HSA) of H_2O_2 (1×10^{-4} M, PBS buffer: pH 7.4), the tubes were put into water bath (37 °C) for 3 h. With the addition of 1, 5-diphenylcarbazide (1.0 mL,



Scheme 1. The structure of $Cr(R\text{-}pic)_3$ complexes 1–8.

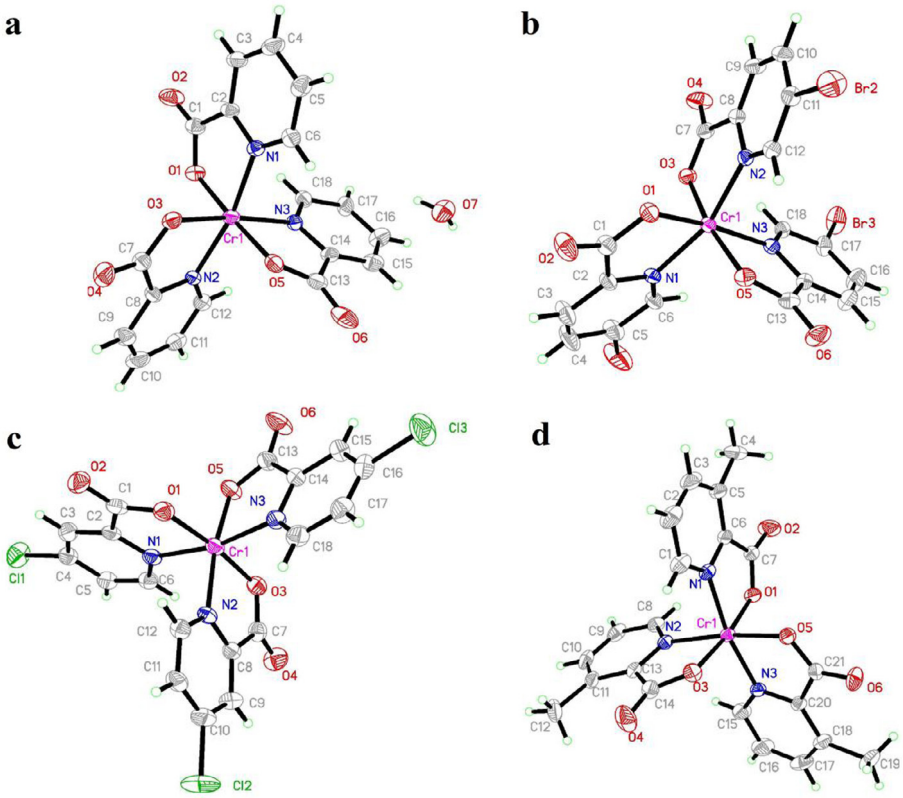


Fig. 1. Crystal structures of complexes **1**(a), **2**(b), **4**(c) and **6**(d).

Table 1
Crystal data and structure refinement for complexes **1** (a), **2** (b), **4** (c) and **6** (d).

	Complex **1**	Complex **2**	Complex **4**	Complex **6**
Empirical formula	C18H14CrN3O7	C18H9Br3CrN3O6	C18H9Cl3CrN3O6	C21H18CrN3O6
Formula weight	436.32	655.01	521.63	460.38
CCDC number		1500350	1500280	1500281
Crystal system	Monoclinic	Monoclinic	Tetragonal	Monoclinic
Space group	C2/c	P2/n	I4 cd	C2/c
a/Å	30.196	9.772	29.632	31.764(3)
b/Å	8.5360	17.776	29.632	8.5367(8)
c/Å	13.9321	13.084	9.316	14.9007(15)
α(°)	90	90	90	90
β(°)	95.0160	110.43	90	96.592(3)
γ(°)	90	90	90	90
V/Å^3	3577.3	2129.8	8179.8	4013.8(7)
Z	8	4	16	8
d g/cm^3	1.620	2.043	1.694	1.524
F(000)	1784	1260	4176	1896
θ	2.48–25.02	2.83–28.26	3.07–28.32	2.86–28.32
h, *k*, *l*	−35/34, −10/10, 6/12	−8/13, −23/23, −17/17	−35/39, −39/37, −12/12	−42/42, −11/7, −19/19
Tot.Uniq.Data R_{int}	8747/3158/0.0635	20070/5251/0.0553	41200/5091/0.1086	19709/4973/0.0334
$N_{reflections}$, $N_{parameter}$	3158/262	5251/280	5091/280	4973/283
GOF on F^2	1.014	1.010	1.029	1.041
R_1[I > 2s (I)],ωR_2	0.0495, 0.1025	0.0451, 0.0875	0.0523, 0.0739	0.0389, 0.0980

0.01 g/mL) and concentrated sulfuric acid (20 μL) to the mixture consecutively, the sample turned to purple from colorless, and Cr (VI) was detected spectrophotometrically at 548 nm. The linear responses were 0.0420–0.0015 L/mg for Cr(VI) [24,25].

2.6. MTT assay

Cell lines at an initial density of 4.5×10^3 cells/well in 96-well plates containing DMEM (180 μL) were cultured for 14 h at first. Followed by another culture for 24 h with the presence of complexes **1**–**8** (20 μL, 1×10^{-3} M, DMSO/buffer, 1:9 V/V). Subsequently, MTT (20 μL, 5 mg/mL, and NaCl buffer) were added and the plates were incubated at 37 °C for 4 h to form a purple formazan. Finally, the medium with MTT was removed from the wells and 200 μL of DMSO was transferred into each well to dissolve the purple formazan. The absorbance at 490 nm was measured with a micro plate reader and absorbance of blank control wells were taken as 100% of viability. Results are indicated as the percentage of viable cells with respect to untreated controls. Values are the mean ± SD of three separate experiments carried

Table 2
Selected bond lengths (Å) and angles (°) of complexes **1**, **2**, **4** and **6**.

		Complex **1**	Complex **2**	Complex **4**	Complex **6**
Bond length/ Å	Cr1−N1	2.048(3)	2.084(3)	2.058(3)	2.0557(2)
	Cr1-N2	2.056(3)	2.057(3)	2.065(3)	2.0594(2)
	Cr1-N3	2.057(3)	2.047(3)	2.042(4)	2.0433(2)
	Cr1-O1	1.954(3)	1.933(3)	1.953(3)	1.9505(1)
	Cr1-O3	1.948(3)	1.948(3)	1.947(3)	1.9368(2)
	Cr1-O5	1.942(3)	1.951(3)	1.936(3)	1.9329(1)
Bond angles (°)	N1-Cr1-N2	168.61(12)	162.69(14)	93.27(13)	92.42(6)
	N1-Cr1- N3	92.22(12)	98.89(13)	167.57(14)	169.84(7)
	N2-Cr1-N3	97.22(12)	95.91(12)	95.85(13)	95.72(6)
	O1-Cr1-N1	80.30(12)	81.10(12)	81.36(14)	79.92(6)
	O1-Cr1-N2	92.53(12)	85.79(12)	94.44(13)	94.32(6)
	O1-Cr1-N3	94.70(12)	170.50(13)	89.52(13)	93.38(6)
	O3-Cr1-N1	90.49(11)	90.64(12)	94.06(14)	95.45(7)
	O3-Cr1-N2	81.17(12)	80.58(12)	80.64(13)	79.88(6)
	O3-Cr1-N3	170.89(12)	89.29(12)	95.74(13)	91.97(7)
	O5-Cr1-N1	99.13(12)	94.47(13)	90.71(13)	92.31(6)
	O5-Cr1- N2	88.69(12)	96.90(13)	171.01(14)	171.74(6)
	O5-Cr1-N3	80.95(12)	80.00(12)	81.49(13)	80.36(6)
	O3-Cr1-O1	94.33(11)	100.20(13)	173.12(13)	172.48(6)
	O5-Cr1-O1	175.60(12)	90.53(13)	94.13(13)	93.18(6)
	O5-Cr1-O3	90.03(12)	168.72(12)	91.04(14)	92.93(6)

out in sextuplicate. DMSO is used as a control comparison, the final concentration of DMSO in the experimental group and DMSO group is limited at 1%.

2.7. Acute toxicity study

All the animal experimental protocols were approved by the Animal centre of First Hospital of Shanxi Medical University (Shanxi Province, China) with the qualified number SCXK (JIN) 2015-0001. Healthy C57 mice at the mass of 20–30 g with equal sex were divided into five groups in this study. DMSO (1%) was used as control, other groups were treated with $CrCl_3$, **1**, and **8**, respectively. On account of insolubility of the complexes in redistilled water and other buffer solution, 1% DMSO was employed as solvent in acute toxicity study. After fasted for 16 h, all mice were administered orally a single dose 1% DMSO, $CrCl_3$, **1** and **8** (10.0 g/kg BW). Observe sostenuto the behaviors of the mice for 1 h, and within 24 h intermittently for every 4 h. Then intragastric administration for 6 days' feeding with the same dosage as administered orally. During the process, all animals are free access to water and food. The toxic symptom and death of all mice need further observation for 1 week [17], and the body weight at 0, 3rd and 7th day should be recorded. At the final stage of the experiment, all the mice were killed by carbon dioxide and dissected after suffering from starvation for 12 h. 3–5 mm sections of liver, kidney and pancreas of the rats stained by hematoxylin and eosin (H&E) were examined macroscopically. The morphological observation of the internal organs was given visually for signs of toxicity [17].

2.8. Hypoglycemic activity study

After 4 weeks feeding with control (normal group) or high-fat (diabetic group) diets: ordinary feed 51.5%, soybean oil 10%, sucrose 20%, lard 15% and cholesterol 3.5%, mice were injected with citric buffer (normal group) or streptozotocin (STZ in citric buffer, pH = 4.4, 50 mg/kg body mass) through tail vein. After continuation of 4 weeks' control or high fat diets, blood samples were drawn and fasting glucose levels (FBG) were determined to confirm induction of diabetes. All mice were starved for 16 h prior treatment, and heparin (0.5 mg/ mL) and NaF (10 mg/mL) were added to the blood sample immediately after the blood was collected. The diagnostic kits, double antibody and antibody coated kits used in this experiment were from Sigma (St. Louis, Mo.) and ICN Biomedicals (Costa Mesa, Calif.), respectively. As expected, FBG levels increase approximately 1-fold following treatment with STZ vs. non-STZ treated mice, and FBG levels $\geq$11.1 mmol/L represents the mice are hyperglycemic.

Fifty mice were randomly divided into one normal group (normal mice) and four diabetic groups (STZ-mice) with 10 mice each group. All the mice were free access to standard solid diet and water during the experimental process. The drug was administrated at the doses of 1000 μg Cr/kg body mass orally once a day for 8 weeks, and the body mass, fasting blood glucose (FBG), fasting serum insulin (FINS), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and high density lipoprotein (LDL) levels of the mice was measured every 4 week thereafter. Blood sample of all mice were obtained through orbital venous plexus [26].

In the end, all the samples were analyzed by Beckman AU5800 automatic biochemical analyzer features in the First Hospital of Shanxi Medical University (Shanxi Province, China). The data were analyzed using statistical software SPSS version 16.0 (SPSS Inc., Chicago, USA) and given as mean ± SD (standard deviation). Experimental results were considered to be specific significant different when $p \leq 0.05$.

3. Results and discussions

3.1. Description of the structure

Complexes $[Cr(pic)_3]\cdot(H_2O)$ (**1**), $Cr(5\text{-Br-pic})_3$ (**2**), $Cr(5\text{-}CF_3\text{-pic})_3$ (**3**), $Cr(4\text{-Cl-pic})_3$ (**4**), $Cr(5\text{-COOH-pic})_3$ (**5**), $Cr(3\text{-}CH_3\text{-pic})_3$ (**6**), $Cr(5\text{-OH-pic})_3$ (**7**) and $Cr(3\text{-OH-pic})_3$ (**8**) were synthesized and characterized by infrared spectroscopy, ESI-MS and elemental analysis. The infrared spectra of the complexes and corresponding ligands are shown in Figs. S5–S12. The FTIR analysis in Table S1 can well depict the coordination between **L1-L8** and Cr^{3+}. For example, stretching vibration of carboxylate for **L2** has been shifted from 1699 to 1677 cm^{-1} after coordination; meanwhile, stretching vibration of –OH in **L2** at 3398 cm^{-1} disappeared in the complex **2**.

The structure of $[Cr(pic)_3]\cdot(H_2O)$ is a mononuclear meridional isomer, and it is almost the same with the literature reported previously [22]. Plenty of O-H···O hydrogen bonds was found in the Crystal packing diagram (Fig. S1). The crystal structure of complexes **2**, **4** and **6** are similar with **1**. $Cr(5\text{-Br-pic})_3$ (**2**) crystallizes in monoclinic *P*2/n space group (Table 1). Central metal Cr(III) ion is chelated by three 5-Br-pic ligands with meridional configuration

and assembles in a distorted octahedron (Fig. 1b). Bond angles of N1-Cr1-N2, O3-Cr1-O5, O1-Cr1-N3 are 162.69, 168.72, 170.50° and the Cr–O/Cr-N distances range from 1.933(3) to 1.951(3) Å and 2.047(3) to 2.084(3) Å (Table 2), respectively. Although no H_2O molecular were found from the crystal packing structure, effective interlayer interactions such as C—H···π interactions was formed (Fig. S2). Cr(4-Cl-pic)$_3$ (**4**) is a mononuclear Cr(III) complex crystallizes in tetragonal I4 cd space group with meridional configuration (Fig. 1c). Cr–O bond lengths ranges from 1.936(3) to 1.953 (3) Å and the bond lengths of Cr–N bonds range from 2.042(4) to 2.065(3) Å. In addition, structure of Cr(3-CH_3-pic)$_3$ (**6**) is shown in Fig. 1d, and it is a meridional isomer as complexes **1**, **2** and **4**. The Cr-N distance ranges from 2.0433 (2) to 2.0594(2) while the Cr-O bond lengths range from 1.9329(1) to 1.9505(2) Å. This is in accordance with the structure of Cr(pic)$_3$ reported in 1992 [22]. Weaker C-H···O interactions exist in intermolecular interactions in **4** and **6** (Figs. S3, S4).

The average Cr-N length is 2.063, 2.055, 2.053 Å, and this is semblable with other similar complexes (2.097 [19], 2.053 [22]) The biggest bond angles of N-Cr-N(#) is 162.69, 167.57 and 169.84 ° for **2**, **4** and **6** respectively, and this is alike with other CrN3O3 complexes (166.75 [27], 174.98 [28] and 168.1° [29]). Overall, there is no significant difference in the bond lengths and bond angles for these complexes although with different substituent group.

Single crystals of complexes **3**, **5**, **7** and **8** could not be obtained, and the molecular structures of these complexes are not definite. We hypothesis that the structures of complexes **3**, **5**, **7** and **8** are also meridional isomers based on the results of UV–vis, ESI-MS and elemental analysis and they were obtained at the same conditions with complexes **1**, **2**, **4** and **6**. Besides, the ESI-MS of the complexes in Figs. S21–S25 of complexes **2**, **3**, **5**, **7** and **8** indentify the formation of Cr complex, respectively.

3.2. Fenton-like reaction

It is reported that free radical ·OH generated by Cr(pic)$_3$ will do harm to chromosome. In order to evaluate the safety of complexes **1**–**8** *in vitro*, ·OH-generation of these complexes were evaluated firstly by traditional Fenton-like reaction in PBS medium, and the absorbance at 532 nm of the samples indirectly reflects the ·OH-generation [20]. As shown in Fig. 2a, ·OH-generation by different complexes is various in identical conditions. In comparison with **1**–**6**, **7** and **8** generate much less ·OH, which may be due to the –OH group on ligands. Perhaps phenolic hydroxyl –OH group is able to capture ·OH, which is confirmed by Fenton-like reaction of ligands alone (Fig. S26). Besides, **2**–**4** generate much more ·OH than **6**, which may result from halogen group according to previous literature [30]. This is because the halogen atom probably occupies the sites that are more easily attacked by ·OH [31,32]. Whatever, all the derivatives generate less **.**OH comparing to Cr(pic)$_3$ and Fe (EDTA).

Apart from PBS medium, Fenton-like reactions were also carried out in HSA (green) and RPMI medium 1640 (blue). As a result in Fig. 2b, complex **8** generate less ·OH than Cr(pic)$_3$ no matter in PBS, 1640 or HSA medium. Interestingly, ·OH-generation by all the samples in RPMI medium 1640 and HSA is far lower than that in PBS medium. The probably reason is that ·OH will be captured and consumed by the bio-reductants or proteins in 1640 or HSA medium. This process is complex and unable to control. To sum up, Cr(III) complexes can generate ·OH with the existence of oxidant and reducing agent by Fenton-like reaction, and ·OH will be consumed by bio-reductants in the medium. Therefore, the damage of ·OH to cells and tissues can be ignored.

3.3. Oxidation reaction

As is known to us all, Cr(VI) is mutagenic agent and exerts potential hazard to organism [33]. To explore the oxidation potential of the Cr(III) complexes, oxidation reaction by hydrogen peroxide in PBS, RPMI medium 1640 and HSA were monitored by UV–vis spectra. The results of oxidation reaction were given in Fig. 3, and the peak appeared at 548 nm reflects the generation of Cr(VI) in the solution. As seen in Fig. 3b, complexes **2**–**4** generate more Cr(VI) than other complexes, and we attribute this to the electronegative group on the ligands (bearing –Br, –CF_3, –Cl). Besides, Cr(VI)-generation of **7** and **8** (bearing -OH group) is least and insusceptible to medium, which is in accordance with the ·OH-generation in Fenton-like reaction. Besides, most of complexes generate much more Cr(VI) in 1640 or HSA medium than that in PBS, and it is because that the HSA or 1640 medium may act as a competitor to make the complexes more easy to decompose and be oxidized. Besides, the reducing agents in HSA and 1640 are able to consume H_2O_2, and the unequal capture ability of H_2O_2 by bio-reductants results in different Cr(VI)-generation in the medium, and this could explain why so big errors in Fig. 3b. As a result of ·OH-generation and Cr(VI)-generation reaction, complexes contain –OH group behaves less *in vitro* toxicity potential than that of halogen group. In addition, the direct oxidation to Cr(VI) by H_2O_2 in cell cultural medium should be paid more attention than Fenton-like reaction for Cr(pic)$_3$ and halogen group complexes.

3.4. MTT assay

Cytotoxicity of complexes **1**–**8** was tested by MTT (3-(4, 5-dimethyl-2-thiazolyl) bromide-2, 5-diphenyl-2H-tetrazolium)

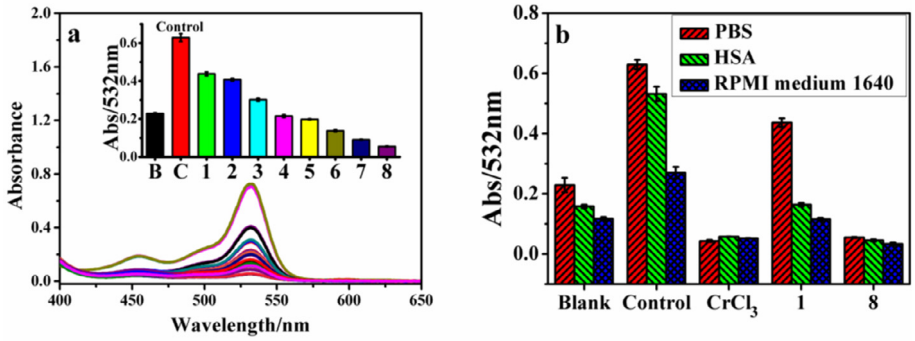


Fig. 2. (a) UV–visible spectra of Cr(III) complexes 1–8 (1.0×10^{-4} M) through Fenton-like reaction in PBS (pH 7.4, 10 mM) (Inset: absorbance at 532 nm of samples). (b) Absorbance at 532 nm of $CrCl_3$, complexes 1 and 8 through Fenton-like reaction in PBS (red), RPMI medium 1640 (green) and HSA (blue). B: Blank group, C: Control group. PBS/HAS/RPMI medium 1640 act as blank group, Fe(EDTA) act as control group.

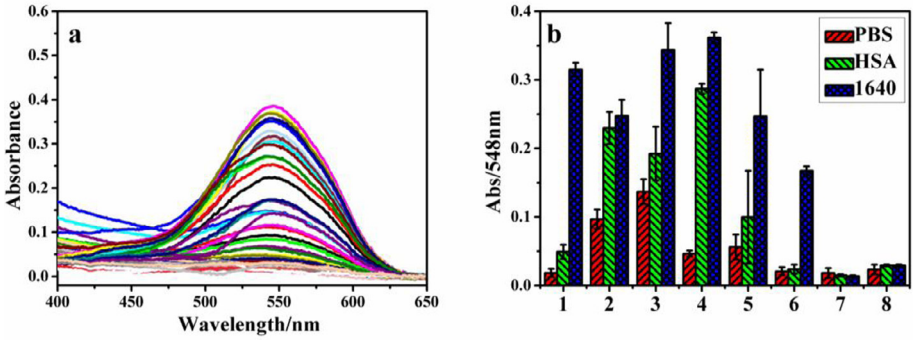


Fig. 3. (a) The UV–visible spectra of complexes 1–8 (1.0×10^{-4} M) oxidized by H_2O_2 (1.0×10^{-4} M, PBS buffer) in PBS/HSA/RPMI medium 1640 with the addition of diphenylcarbazide (0.01 g/mL) and concentrated sulfuric acid. (b) Absorbance at 548 nm of UV–visible spectra in Fig. 3a (red: PBS, green: HSA, blue: RPMI medium 1640). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assay against MCF-7 cells, and this method was based on the theory that MTT can be reduced by mitochondrial dehydrogenases of living cells to purple formazan. The formazan formed by living cell is soluble in DMSO with maximum absorbance at 490 and 570 nm. The result was given in Fig. 4, and the corresponding ligands were also employed as a control experiment (Fig. S27). Considering the impact of DMSO, cell viability of all samples attained to 90% although cell viability of **1**–**6** is slightly lower than that of **7** and **8**. That's to say, all complexes behave hypotoxic to cells lines within 24 h, and the result is well consistent with ·OH-generation and Cr(VI)-generation reaction in 1640 or HSA medium. Therefore, Cr(III) complexes is nearly non-toxic to cell lines and the worry about Cr(VI)-generation in cells should be ignored [17].

3.5. Acute toxicity study

To verify the acute toxicity of these Cr(III) complexes, **1** and **8** was selected as experimental group and $CrCl_3$ was used as a control group. All mice survived to the end and no significant effects of the complexes on growth or final mean body mass of mice were observed (Table S2, Fig. S28) after administration of Cr(III) complexes (10.0 g/kg BW) and DMSO (1%) by gavage, and the microanatomical features of representational tissue slices were given as Fig. 5. There was no distinct disparity noticed in body mass between the experimental group and control group (Fig. S28 and Table S2), and there seems to be no damage in kidney, liver and pancreas tissue from the outer appearance (Fig. 5). Kidney

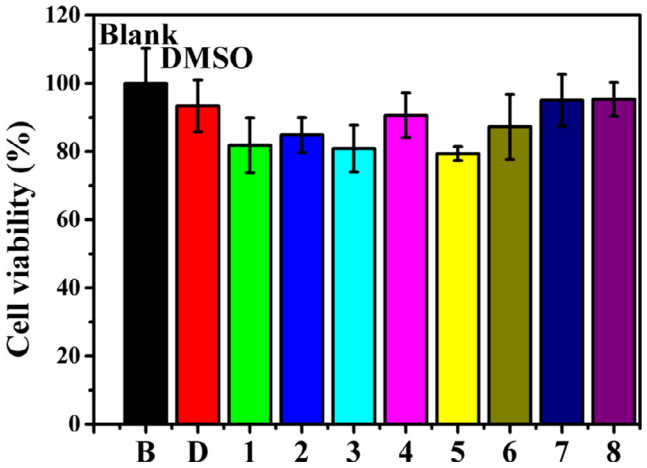


Fig. 4. Effect of the complexes 1–8 (1.0×10^{-4} M) on viability of MCF-7 cells (4500), B: blank, D: DMSO.

histology sections of C57 rats treated with Cr(III) compounds showed normal structures. No adverse effects were found on the histoarchitecture of hepatocytes after administered acutely. All of them revealed that high-dose treatment with of Cr(III) compounds exhibit low toxicity or non-toxic to mice in this period, and this is accordant with the literature reports [4].

It has been reported by in extension reviews that the toxic effects of $Cr(pic)_3$ arises only in situations, for example, when the special contact molecule is present in cell culture, injection into mammals and animals with simple digestive systems such as fruit flies); in mammals, the compound degrades in the stomach and is non-toxic [34].

3.6. Anti-hyperglycemic activity study

To assess the therapeutic effects of synthesized complexes on diabetes, the hypoglycemic activity of complex **8** on STZ-mice was examined for 2 months due to it minimum potential hazards during ·OH and Cr(VI) generation experiments. The FBG, FINS, TC, TG, HDL and LDL levels of the lean control mice and STZ-mice were monitored after oral gavage of $CrCl_3{\cdot}6H_2O$, **1** and **8** at 0, 4th and 8th week (Fig. 6). No significant change in physical activities occurred on the administration of both complex, and all the mice survived to the end.

FBG (6.07 ± 0.92 mmol/L), FINS (12.36 ± 1.50 μmol/L), TC (1.92 ± 0.15 mmol/L) and TG (0.55 ± 0.05 mmol/L) levels of mice in control group are in the normal range, while the mice in diabetic control group are significantly ($p < 0.05$) higher than those of the control group during the experimental process (Table 3). FBG levels for the diabetic control group is more than 11.1 mmol/L representing the mice are hyperglycemic. Meanwhile, HFD significantly affects other parameters for the diabetic control group.

In comparison with diabetic control group, the FBG levels decreased significantly after administration with $CrCl_3{\cdot}6H_2O$ and complex **1** after 4 weeks ($p < 0.05$). FBG levels of the mice decreased 27.42% and 26.23% for $CrCl_3$ and complex **1** groups after 8 weeks, while complex **8** only led to a slightly decrease (10.26%) of FBG levels after 8 weeks. Even so, the FBG levels continued to be much higher than normal mice after treatment with complexes **1** and **8** for 8 weeks (5.60 vs. ≥9.42 mmol/L, Fig. 6a).

FINS levels for $CrCl_3{\cdot}6H_2O$ group decreased 12.98% after 4 weeks and the decrease was abolished for 8 weeks (Fig. 6b). While FINS levels of 1 and 8 treated mice remained no obvious decrease and they were much higher than that of normal group. TC levels decreased 18.22% and 1.80% for $CrCl_3{\cdot}6H_2O$ and **1** group after 8 weeks treatment, respectively. No significant changes were observed for TG levels in these three Cr(III) complexes group (Fig. 6c and d). The decrease of FBG, FINS and TC for $CrCl_3{\cdot}6H_2O$

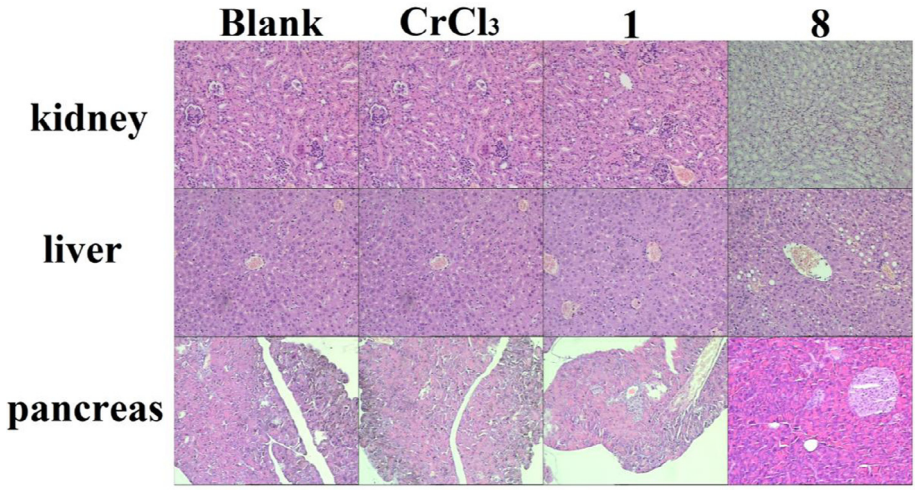


Fig. 5. Effects of $CrCl_3$, complexes 1 and 8 on kidney, liver and pancreas tissue slices of the mice in acute toxicity studies. Control group: DMSO (1%). Experimental group: $CrCl_3$, **1** and **8** (10.0 g/kg BW).

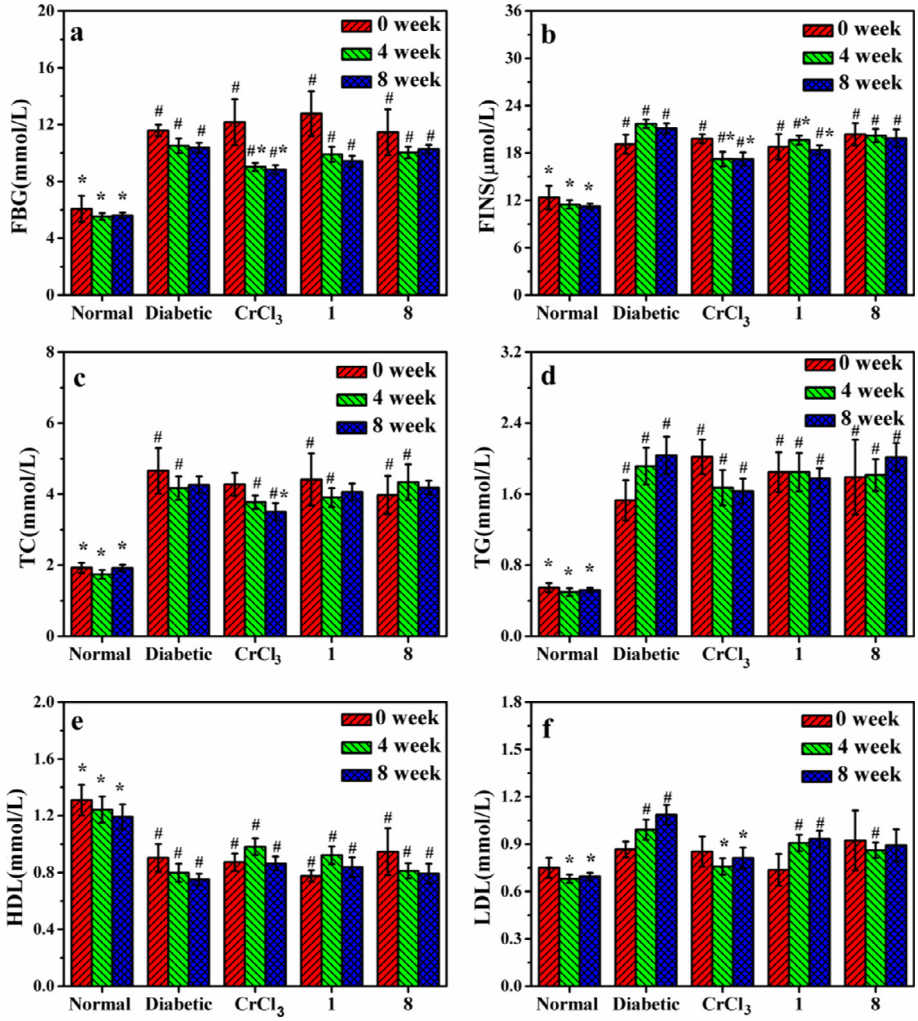


Fig. 6. Effect of Cr supplementation on fasting blood glucose (FBG, mmol/L) (a), fasting serum insulin (FINS, μmol/L) (**b**), Total cholesterol (TC, mmol/L) (**c**), Triglyceride (TG, mmol/L) (**d**), high density lipoprotein (HDL, mmol/L) and low density lipoprotein (LDL, mmol/L) of mice. Rats were supplemented with control buffer, $CrCl_3$, **1** or **8** (1000 μg Cr/kg body mass) by gavage daily for 8 weeks. #: diabetic group versus normal control ($p < 0.05$). *: other group versus diabetic group ($p < 0.05$).

Table 3
Alter of body mass, FBG, FINS, TC, TG HDL and LDL levels of control group and the mice followed administration with $CrCl_3$, 1 and 8 carrier (control) by oral gavage during anti-hyperglycemic activity study. #: diabetic group versus normal control ($p < 0.05$). *: other group versus diabetic group ($p < 0.05$).

	Week	Normal rats	Diabetic rats	$CrCl_3$ treated rats	**1**-treated rats	**8**-treated rats
BM/g	0	19.24 ± 1.16	23.62 ± 2.05	24.14 ± 2.14	23.39 ± 1.93	23.32 ± 1.96
	4	22.45 ± 1.55	30.5 ± 2.40	29.42 ± 1.62	29.36 ± 1.96	29.51 ± 0.82
	8	29.35 ± 1.48	40.82 ± 2.18	37.89 ± 3.01	39.75 ± 1.72	39.91 ± 2.23
FBG/mmol·L^{-1}	0	6.07 ± 0.92*	11.59 ± 0.39#	12.18 ± 1.61#	12.77 ± 1.57#	11.47 ± 1.62#
	4	5.54 ± 0.23*	10.50 ± 0.52#	9.02 ± 0.28#*	9.91 ± 0.52#	10.03 ± 0.40#
	8	5.60 ± 0.20*	10.37 ± 0.35#	8.84 ± 0.30#*	9.42 ± 0.38#	10.26 ± 0.31#
FINS/μmol·L^{-1}	0	12.36 ± 1.50*	19.13 ± 1.21#	19.80 ± 0.54#	18.78 ± 1.60#	20.37 ± 1.41#
	4	11.45 ± 0.52*	21.71 ± 0.52#	17.23 ± 0.91#*	19.64 ± 0.58#*	20.22 ± 0.85#
	8	11.25 ± 0.34*	21.11 ± 0.66#	17.22 ± 0.86#*	18.40 ± 0.58#*	19.89 ± 1.11#
TC/mmol·L^{-1}	0	1.92 ± 0.15*	4.66 ± 0.64#	4.28 ± 0.32#	4.41 ± 0.74#	3.97 ± 0.54#
	4	1.74 ± 0.12*	4.17 ± 0.32#	3.77 ± 0.19#	3.90 ± 0.26#	4.34 ± 0.50#
	8	1.92 ± 0.093*	4.26 ± 0.23	3.50 ± 0.24#*	4.06 ± 0.23	4.18 ± 0.19#
TG/mmol·L^{-1}	0	0.55 ± 0.050	1.53 ± 0.22	2.02 ± 0.19	1.85 ± 0.22	1.79 ± 0.42
	4	0.50 ± 0.043*	1.92 ± 0.21#	1.67 ± 0.20*	1.85 ± 0.22#	1.81 ± 0.18#
	8	0.52 ± 0.026*	2.04 ± 0.21#	1.63 ± 0.14*	1.78 ± 0.11#	2.02 ± 0.16
HDL/mmol·L^{-1}	0	1.31 ± 0.11*	0.90 ± 0.099#	0.87 ± 0.062#	0.78 ± 0.040#	0.95 ± 0.16#
	4	1.24 ± 0.092*	0.80 ± 0.064#	0.98 ± 0.059#	0.92 ± 0.061#	0.81 ± 0.053#
	8	1.19 ± 0.086*	0.75 ± 0.040#	0.86 ± 0.049#	0.84 ± 0.070#	0.79 ± 0.069#
LDL/mmol·L^{-1}	0	0.75 ± 0.064	0.87 ± 0.050	0.85 ± 0.095	0.74 ± 0.10	0.92 ± 0.19
	4	0.68 ± 0.027*	0.99 ± 0.064#	0.76 ± 0.052*	0.91 ± 0.052#	0.86 ± 0.050#
	8	0.70 ± 0.024*	1.09 ± 0.062#	0.81 ± 0.066*	0.93 ± 0.052#	0.89 ± 0.10

group is bigger than that of **1** and **8** groups. It may be due to the inert activity of complexes **1** and **8** making Cr(III) difficult to be decomposed and absorbed.

Moreover, the HDL, LDL levels of mice was also examined in STZ-mice for 56 days. As seen in Fig. 6e-f, HDL and LDL levels of all the Cr(III) complexes group remained changeless throughout the administration. Besides, all drugs have no obvious decrease on the body mass of the mice (Fig. S29), and this is consistent with the literature reported previously [8].

4. Conclusion

In an attempt to improve the safety and biological activity of Cr$(pic)_3$, seven new analogues were obtained through pic ligands with different substituent groups. The structure, Fenton-like reaction, oxidation reaction, cytotoxicity, acute toxicity and hypoglycemic activity of all the complexes are evaluated. It was found that the substituent groups affected the physicochemical activities of the complexes such as ·OH- and Cr(VI)-generation in tube. For example, halogen group complexes generate more ·OH and Cr(VI) than complexes with –OH group, however, these differences can be neglected in cell and animal experiments. This is attributed to the lower concentration of oxidant but high concentration of bio-reductants *in vivo*. This concept was verified by cell MTT assay and acute toxicity study: no obvious cellular damage and tissue injury were observed for Cr$(pic)_3$ and its derivatives. On the other hand, hypoglycemic activity study indicated that Cr$(R\text{-}pic)_3$ complexes including Cr$(pic)_3$ have not significant influence than $CrCl_3 \cdot 6H_2O$ salt on the blood glucose, serum insulin, total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein of diabetic mice. Once again this result questioned the widely use of Cr$(pic)_3$ as a validity nutrition additives.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2017.05.041.

References

[1] K. Schwarz, W. Mertz, Arch. Biochem. Biophys. 85 (1959) 292–295.
[2] J.B. Vincent, Biol. Trace Elem. Res. 166 (2015) 7–12.
[3] H. Wang, A. Kruszewski, D.L. Brautigan, Biochemistry 44 (2005) 8167–8175.
[4] A. Levina, T.H. Pham, P.A. Lay, Angew. Chem. Int. Ed. 55 (2016) 8104–8107.
[5] J.B. Vincent, Polyhedron 20 (2001) 1–26.
[6] J.K. Speetjens, R.A. Collins, J.B. Vincent, S.A. Woski, Chem. Res. Toxicol. 12 (1999) 483–487.
[7] J.B. Vincent, Met. Ions Life Sci. 13 (2013) 171–198.
[8] M.D. Stout, A. Nyska, B.J. Collins, K.L. Witt, G.E. Kissling, D.E. Malarkey, M.J. Hooth, Food Chem. Toxicol. 47 (2009) 729–733.
[9] Z. Liu, A. Habtemariam, A.M. Pizarro, S.A. Fletcher, A. Kisova, O. Vrana, L. Salassa, P.C. Bruijnincx, G.J. Clarkson, V. Brabec, P.J. Sadler, J. Med. Chem. 54 (2011) 3011–3026.
[10] C. Wang, J. Liu, Z. Tian, M. Tian, L. Tian, W. Zhao, Z. Liu, Dalton Trans. (2017).
[11] M.R. Kandadi, M.K. Unnikrishnan, A.K. Warrier, M. Du, J. Ren, N. Sreejayan, J. Inorg. Biochem. 105 (2011) 58–62.
[12] H.G. Preuss, B. Echard, D. Clouatre, D. Bagchi, N.V. Perricone, J. Inorg. Biochem. 105 (2011) 1344–1349.
[13] S.K. Jain, G. Kahlon, L. Morehead, R. Dhawan, B. Lieblong, T. Stapleton, G. Caldito, R. Hoeldtke, S.N. Levine, P.F. Bass 3rd, Mol. Nutr. Food Res. 56 (2012) 1333–1341.
[14] S.K. Jain, J.L. Croad, T. Velusamy, J.L. Rains, R. Bull, Mol. Nutr. Food Res. 54 (2010) 1371–1380.
[15] J.B. Vincent, J. Am. Coll. Nutr. 18 (1999) 6–12.
[16] M.M. Abdel-Monem, M.D. Anderson, in: U.S. Patent (Ed.), Zinpro Corporation, United States, 2007, pp. 1–7.
[17] H.Y. Tang, Q.G. Xiao, H.B. Xu, Y. Zhang, J. Trace Elem. Med. Biol. 29 (2015) 136–144.
[18] B. Liu, J. Chai, X.Q. Hu, Y.J. Zhang, J.X. Nan, B.S. Yang, Inorg. Chem. Commun. 52 (2015) 27–30.
[19] G.H. Xu, J.F. Ma, Y.Y. Liu, S.L. Li, Acta Crystallogr., Sect. C 62 (2006) m581–m583.
[20] V. Nair, G.A. Turner, Lipids 19 (1984) 804–805.
[21] I. Mulyani, A. Levina, P.A. Lay, Angew. Chem. Int. Ed. 43 (2004) 4504–4507.
[22] D.M. Steams, W.H. Armsbong, Inorg. Chem. 31 (1992) 5178–5184.
[23] G.M. Sheldrick, Acta Crystallogr. A 64 (2008) 112–122.
[24] J.E.T. Andersen, Anal. Chim. Acta 361 (1998) 125–131.
[25] M. Pettine, T. La noce, A. Liberatori, L. Loreti, Anal. Chim. Acta 209 (1988) 315–319.
[26] F. Li, X. Wu, Y. Zou, T. Zhao, M. Zhang, W. Feng, L. Yang, Food Chem. Toxicol. 50 (2012) 1623–1631.
[27] N. Clement, C. Toussaint, G. Rogez, C. Loose, J. Kortus, L. Brelot, S. Choua, S. Dagorne, P. Turek, R. Welter, Dalton Trans. 39 (2010) 4579–4585.
[28] V.C. Gibson, C. Newton, C. Redshaw, G.A. Solan, A.J.P. White, D.J. Williams, Dalton Trans. (2003) 4612–4617.

[29] H.G. Raubenheimer, A. du Toit, M. du Toit, J. An, L. van Niekerk, S. Cronje, C. Esterhuysen, A.M. Crouch, Dalton Trans. (2004) 1173–1180.
[30] Y.Y. Zhang, Q.X. Zhou, Y. Zheng, K. Li, G.Y. Jiang, Y.J. Hou, B.W. Zhang, X.S. Wang, Inorg. Chem. 55 (2016) 4296–4300.
[31] N. Selvarajan, N.V. Raghavan, J. Phys. Chem. 84 (1980) 2548–2551.
[32] M. Yamaguchi, J. Phys. Chem. A 115 (2011) 14620–14628.
[33] J.M. Chen, O.J. Hao, Crit. Rev. Environ. Sci. Tec. 28 (2010) 219–251.
[34] J.B. Vincent, Toxicology of chromium(III), in: The Bioinorganic Chemistry of Chromium, John Wiley & Sons, Ltd, 2012, pp. 195–213.