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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.

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

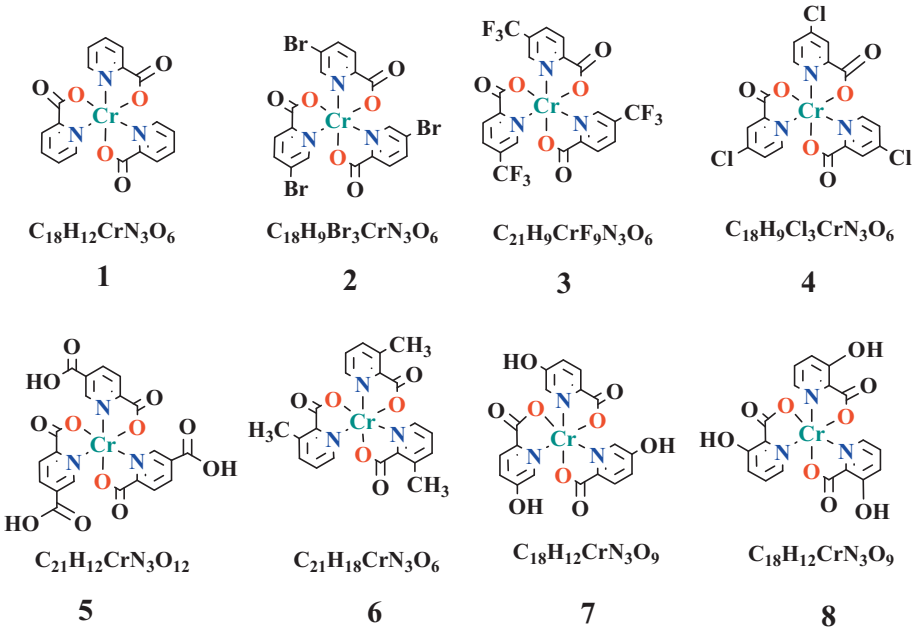
The XRD measurements for complexes **1**, **2**, **4**, and **6** were carried out with a CCD detector equipped with graphite-monochrome Mo Kα radiation. The structures of all complexes mentioned above were obtained by direct methods with SHELXL-97 and refined by full-matrix least-squares techniques 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.