Inorganica Chimica Acta 466 (2017) 478–485

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

L-Tryptophan Schiff base cadmium(II) complexes as a new class of proteasome inhibitors and apoptosis inducers in human breast cancer cells

Nan Zhang [a,b,c], Yuhua Fan [a], Guimei Huang [a], Daniela Buac [b], Caifeng Bi [a,*], Yanfei Ma [c], Xinping Wang [c], Zhen Zhang [a], Xia Zhang [a], Q. Ping Dou [b,*]

[a] Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, College of Chemistry and Chemical Engineering, Ocean University of China, 238 Songling Road, Qingdao, Shandong 266100, China
[b] Barbara Ann Karmanos Cancer Institute, Departments of Oncology, Pharmacology and Pathology, School of Medicine, Wayne State University, 540.1 HWCRC, 4100 John R Road, Detroit, MI 48201, USA
[c] Key Laboratory of Eco-chemical Engineering, Ministry of Education, College of Marine Science and Biological Engineering, Qingdao University of Science and Technology, 53 Zhengzhou Road, Qingdao, Shandong 266042, China

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 29 June 2017
Accepted 4 July 2017
Available online 8 July 2017

Keywords:
L-Tryptophan
Cadmium Schiff base complex
Proteasome inhibitor
Apoptosis

ABSTRACT

Although cadmium is a widespread environmental contaminant and human carcinogen, our studies indicate an organic cadmium complex to be a potent inhibitor of proteasomal chymotrypsin-like (CT-like) activity, further capable of inducing apoptosis in a cancer cell-specific manner. Many clinical studies suggest the use of proteasome inhibitors as potential novel anticancer agents and in the present study, we have synthesized three novel L-tryptophan-containing cadmium complexes: $Cd(C_{17}H_{15}N_4O_2)_2{\cdot}2CH_3OH$ (**1**) ($C_{17}H_{15}N_4O_2$ = 2-acetylpyrazine-L-tryptophan), $Cd(C_{17}H_{15}N_2O_3)_2{\cdot}2CH_3OH$ (**2**) ($C_{17}H_{15}N_2O_3$ = 5-methylfurfural-L-tryptophan) and $Cd(C_{16}H_{12}N_2O_2SBr)_2{\cdot}2CH_3OH$ (**3**) ($C_{16}H_{12}N_2O_2SBr$ = 5-bromo-2-thiophenecarbaldehyde-L-tryptophan) and found that under comparable conditions cadmium complexes **1** and **2**, but not **3**, have proteasome-inhibitory activity in human breast cancer MDA-MB-231 cells. Our results suggest that L-tryptophan Schiff base cadmium complexes are potent proteasome inhibitors.

1. Introduction

The ubiquitin-proteasome pathway, which plays a significant role in regulating cell proliferation and cell death [1,2], has been widely studied in human cancers. The proteasome is a massive multicatalytic protease responsible for degrading a large number of cellular proteins. These target proteins are first tagged with ubiquitin, and then sent to the 26S proteasome for destruction. The 20S proteasome, the core of the 26S proteasome complex, contains at least three distinct catalytic activities, including chymotrypsin-like activity [3,4]. Several studies have shown that inhibition of the proteasomal chymotrypsin-like activity results in the accumulation of several target proteins, such as IκB-α, Bax and p27, and induction of apoptosis in various types of tumor cells [5–7]. Our laboratory has studied a number of the metal-based drugs, including organic copper-, zinc-, and gold-based complexes, all of which are capable of inhibiting the chymotrypsin-like activity of the proteasome, followed by the induction of apoptosis *in vitro* and *in vivo* [8–12].

Cadmium is often classified as a widespread environmental contaminant and human carcinogen. It has recently been proposed that cadmium ingestion may increase the risk of developing breast cancer [13]. However, some literature does point to cases where cadmium has been shown to affect cell proliferation, differentiation and apoptosis. Studies have shown that cadmium can induce p53-dependent apoptosis and down-regulation of the x-linked inhibitor of apoptosis protein (XIAP) in human prostate cancer cells [14]. It also has an effect on p38/MAPK isoforms [15] and plays an important role in the promotion of breast cancer cell growth by potentiating the interaction between ERα and c-Jun [13]. In contrast, some reports link cadmium exposure to genomic instability, aberrant gene expression, and inhibition of DNA damage repair and apoptosis through complex and multifactorial mechanisms [16,17]. So we asked the question: is cadmium just a causal factor in these studies, or does it really possess the potential to inhibit cancer cell proliferation? The answer and the possible mechanisms remain unknown. On the other hand, because of high anticancer

* Corresponding authors.
E-mail addresses: bicaifeng301@163.com (C. Bi), doup@karmanos.org (Q.P. Dou).

http://dx.doi.org/10.1016/j.ica.2017.07.006
0020-1693/

activity, some Schiff bases and Schiff base complexes derived from heterocyclic moieties became the hot research topics for years [18–20]. A preliminary examination was reported by us that the 2-acetylpyridine-L-tryptophan cadmium complex can act as a potent proteasome inhibitor and further induce apoptosis [21]. So we hypothesized that synthetic forms of cadmium with heterocycle-L-tryptophan Schiff base ligands may have cancer-specific proteasome-inhibitory and apoptosis inducing activities. To test this hypothesis, three novel cadmium-containing complexes: Cd($C_{17}H_{15}N_4O_2$)$_2$·2CH_3OH (**1**) ($C_{17}H_{15}N_4O_2$ = 2-acetylpyrazine-L-tryptophan), Cd($C_{17}H_{15}N_2O_3$)$_2$·2CH_3OH (**2**) ($C_{17}H_{15}N_2O_3$ = 5-methylfurfural-L-tryptophan) and Cd($C_{16}H_{12}N_2O_2SBr$)$_2$·2CH_3OH (**3**) ($C_{16}H_{12}N_2O_2SBr$ = 5-bromo-2-thiophenecarbaldehyde-L-tryptophan) (Fig. 1A) with different heterocycle-L-tryptophan Schiff base structures as ligands have been synthesized and characterized by IR, UV, elemental analysis, ^{1}H NMR analysis, thermogravimetric analysis and molar conductivity analysis. The ability of these compounds to inhibit proliferation and induce apoptosis in MDA-MB-231 breast cancer cells was examined, focusing on direct comparison of **2** and **3**.

2. Materials and methods

All of the chemicals were used without further purification for this work. 2-Acetylpyrazine, 5-methylfurfural and 5-bromo-2-thiophenecarbaldehyde were all purchased from Acros and L-tryptophan was purchased from Aladdin. The chemical agents, DMSO and 3-(4, 5-dimethyltiazol-2-yl)-2.5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). All compounds were made as 50 mM stocks in DMSO and stored at 4 °C. DMEM/F12 (1:1) and penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Aleken Biologicals (Nash, TX, USA). The fluorogenic peptide substrate Suc-LLVY-AMC (for the CT-activity assay) was purchased from Calbiochem (San Diego, CA). Rabbit polyclonal antibody against human poly (ADP-ribose) polymerase (PARP) (H-250) was purchased from BD Bioscience Pharmingen (San Diego, CA). Mouse monoclonal antibodies against ubiquitin (P4D1) and IκB-α (H-4), goat polyclonal antibody against β-actin (C-11) and all secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA).

Elemental analyses were carried out on a Carlo Erba 1106 full-automatic trace organic elemental analyzer. Infrared spectra were recorded as KBr pellets on a Nicolet 170SX spectrophotometer in the 4000–400 cm^{-1} region. The UV spectra were performed on a Unicam UV2 spectrometer. ^{1}H NMR spectra were obtained on a Bruker AVANCE III (600-MHz) spectrometer. Thermogravimetric measurements were made using a Perkin-Elmer TGA7 instrument. The amounts of three cadmium complexes were 8.348 mg, 7.263 mg and 7.418 mg, respectively, and the type of crucible for analysis was high purity alumina crucibles (+ lids). The heating rate was programmed to be 10 °C min^{-1} with a protecting stream of N_2 flowing at a rate of 40 mL min^{-1}. Molar conductivity was

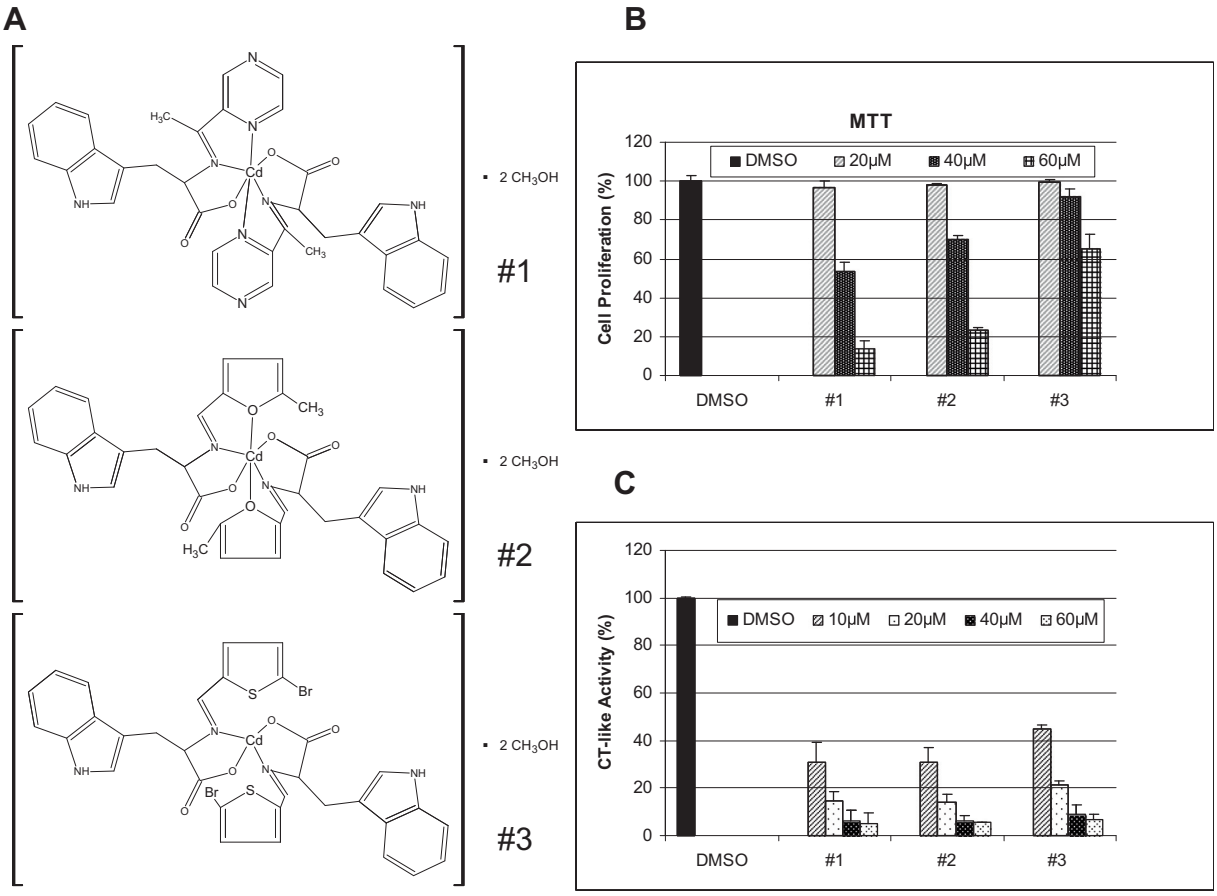

Fig. 1. Chemical structures of cadmium complexes **1**, **2** and **3** (A). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of MDA-MB-231 cells treated with **1**–**3** (B): MDA-MB-231 cells were treated with each cadmium complex for 24 h at various concentrations as indicated. After 24 h, the medium was removed, and the cells were treated with MTT solution, as described in "Experimental" Dimethyl sulfoxide (DMSO) was used as a control. Inhibition of cell-free proteasome (C): Cell-free proteasome (10 μg) was incubated with DMSO or various concentrations of **1**–**3** for 2 h, followed by proteasomal chymotrypsin-like activity assay.