Research Article

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Construction of a new luminescent Cd(II) compound for the detection of Fe³⁺ and treatment of Hepatitis B

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Abstract: A novel porous Cd(II) metal–organic framework (MOF) with the chemical composition of {[H₃O][Cd(bci)]}_n·2n (H₂O) (**1**, H₃bci = bis(2-carboxyethyl)isocyanurate) was prepared via hydrothermal responses of H₃bci and Cd(NO₃)₂·4H₂O. Luminescent property investigations indicated that compound **1** had high sensitivity toward Fe³⁺, and the detection limit of Fe³⁺ was as low as 2.15 × 10^{−4} M. The application values of Fe³⁺ on Hepatitis B were assessed, and its associated mechanism was identified simultaneously. First, the cytotoxic mediator (TNF-α) substance released into plasma was measured through the ELISA. In addition, real-time RT-PCR was employed to determine the Hepatitis B viral replication gene relative expression levels. Finally, the novel compound's toxicity against human normal liver cells was tested by using the CCK-8 assay.

Keywords: Cd(II) compound, porous framework, luminescence sensing, Hepatitis B, toxicity

1 Introduction

Hepatitis B infection can cause acute and chronic Hepatitis B, thereby resulting in chronic liver diseases, for instance, hepatocellular carcinoma and cirrhosis [1]. It is an important infectious disease that seriously affects the health of people. Although the antiviral treatment of interferon and nucleoside analogs has a certain effect in some Hepatitis B

patients, the comprehensive treatment of combined traditional Chinese and Western medicine has also received certain results in the end-stage treatment of liver disease, but its effect is not ideal [2,3]. Therefore, new technologies and new methods for treating the Hepatitis B must be explored.

In the last ten years, metal–organic frameworks (MOFs) have drawn extensive research attention because of their structural diversities and their application prospects as the functional materials in the areas of luminescent probe, non-linear optics, photocatalysis, gas adsorption, and magnetism [4–7]. At present, establishing the luminescent MOFs for selectively determining the organic small molecules, heavy metal ions, and aromatic explosives has received particular interest [8–11]. Compared with traditional detection techniques, MOF-based luminescent probes show some advantages such as high selectivity, high sensitivity, quick response, naked-eye observability, and simple operation [12,13]. Therefore, numerous luminescent MOFs have been synthesized and served as luminescent probes for exploring, Fe³⁺, and ions in the water [14–17].

With the development of the modern industry, many harmful substances, including nitroaromatic explosives, volatile organic solvent molecules, and heavy metal ions, have been discharged directly into the environment, which would lead to the sequence of harm to ecological environment and the health of human [18]. In protecting human safety and ecological balance, developing a new MOF-based luminescent probe with high selectivity together with high sensitivity is essential for determining these harmful substances. Herein, we reported on the generation along with characterization of a fresh 3-dimensional porous Cd(II) MOF, namely, {[H₃O][Cd(bci)]}_n·2n(H₂O) (**1**, H₃bci = bis(2-carboxyethyl)isocyanurate). The luminescence studies demonstrated that the **1** could be employed as a luminescent probe for the high sensitivity Fe³⁺ ion, and the detection limit of Fe³⁺ was calculated to be as low as 2.15 × 10^{−4} M. Furthermore, the compound's treatment activity against Hepatitis B was investigated, and in meantime, the corresponding mechanism was studied.

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2 Experimental

2.1 Materials and instrumentation

$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (98%+, AR) was obtained from Shanghai Titan Scientific Company. H_3bci (97%, AR) ligand was supplied by Jinan Henghua Chemical Reagent Company. Dimethylformamide (DMF; 99.99%, AR) was brought from Shanghai Guoyao Chemical Reagent Company. In order to investigate the elements of Carbon, Nitrogen together with Hydrogen, Vario EL III was employed. PANalytical X'Pert Pro was applied to explore PXRD, in which the step size of $\text{Cu}/\text{K}\alpha$ radiation (where λ is 1.54056 Å) is 0.05°. With the temperature from 30 to 800°C, by utilizing NETSCH STA-449C, TGA was finished with 0°C per min increasing rate under N flow. FLS920, an Edinburgh Analytical instrument, was applied to collect luminescent spectra.

2.2 Synthesis of compound

$\{[\text{H}_3\text{O}][\text{Cd}(\text{bci})]\}_n \cdot 2n(\text{H}_2\text{O})$ (1)

We sealed the mixture created through 0.1 mmol H_3bci , 0.100 mmol $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.0 mL H_2O , and 1 mL DMF into a tiny glass bottle (20.0 mL), which was then placed for two days at 110°C temperature. Next, cooling this complex with 2°C per min decreasing rate at ambient temperature, we acquired the colorless massive crystals of the compound **1** with 38 percent yield in accordance with H_3bci . Elemental analysis Calcd. for the $\text{C}_9\text{H}_{14}\text{CdN}_3\text{O}_{10}$: N, 9.62, C, 24.74, and H, 3.21% found: N, 9.64, C, 24.78, and H, 3.19%.

2.3 X-ray crystallography

The single-crystal information of compound **1** was gathered using an Oxford Xcalibur E controlled through computer, in which the T of graphite-monochromated $\text{Cu}-\text{K}\alpha$ radiation (λ is equal to 0.71073 Å) is equal to 293(2) K. The construction of compound **1** was determined through the dual direct approach and improved through the full-matrix least square approach according to F^2 employing *SHELXL*-2014 [19]. The lattice water molecules were squeezed out via the PLATON program [20]. Crystallographic information of **1** is shown in Table 1. Compound **1**'s selected angles (°) and bond lengths (Å) are enumerated in Table S1.

2.4 Sensing experiments

Two milligrams of finely ground specimens of compound **1** were separately disseminated into 2 mL of $\text{M}(\text{NO}_3)_n$ in

Table 1: The complex **1**'s crystal information

Formula	$\text{C}_9\text{H}_{14}\text{CdN}_3\text{O}_{10}$
Fw	436.59
Crystal system	orthorhombic
Space group	<i>Pnma</i>
<i>a</i> (Å)	8.0632(3)
<i>b</i> (Å)	13.4254(6)
<i>c</i> (Å)	14.4599(6)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å ³)	1565.31 (11)
<i>Z</i>	4
Density (calculated)	1.623
Abs. coeff. (/mm)	11.489
Total reflections	4,561
Unique reflections	1,700
Goodness of fit on F^2	1.161
Final <i>R</i> indices [$I > 2\sigma(I)$]	$R = 0.0384$, $wR_2 = 0.1036$
<i>R</i> (all data)	$R = 0.0399$, $wR_2 = 0.1048$
CCDC	2081898

water (0.01 mol/L, $\text{M} = \text{Al}^{3+}$, Pb^{2+} , Co^{2+} , Hg^{2+} , Na^+ , Cu^{2+} , Ni^{2+} , Ag^+ , and Fe^{3+}), and the above-mentioned suspensions were treated ultrasonically for half an hour. Next, these suspensions were maintained in 1 cm wide quartz cell to carry out fluorescence determination. The titration experiments of Fe^{3+} ions were performed as previously described by the gradual addition of Fe^{3+} concentration.

2.5 ELISA detection kit

After several trials, we found that the as-created Cd-MOF was insolvable in ordinary solvents; as a result, its stock solution was applied for the below biological activity detections, which were well-documented in the literature [19,20]. First, about 150–160 mg of the created MOF was stored in mortar and then they were manually ground with the pestle for half an hour to acquire the fine powders. Next, the above powders were immersed in 50 mL dimethyl sulfoxide and were then sonicated at 70 W for two hours to gather the well-dispersed stock solution. We used the ELISA detection kit in the current research to identify the levels of TNF- α liberated into the plasma following the compound treatment. The process was performed following the instructions with slight modification. In brief, fifty Balb/c mice were offered via Nanjing University's Model Animal Center, which were utilized in the current research. The entire conduction was granted through the Nanjing University's Animal Ethics Committee. The Hepatitis B virus was used to infect the

animal for generating the animal model of Hepatitis B. For the next treatment, the mixture was treated at 1, 2, and 5 mg/kg concentration. The TNF- α content released into plasma after conducting the compound treatment was measured with ELISA.

2.6 Real-time RT-PCR

In this investigation, to explore the inhibitory activity of this new complex on the relative expression of the Hepatitis B viral replication gene, we applied real-time RT-PCR assay. Hepatitis B virus was collected and subsequently treated by this compound with 10, 20, and 50 ng/mL concentration. Next, the Hepatitis B virus was harvested, and TRIzol Reagent was applied to extract the overall RNA under the manufacturer's instruction. Its concentration was measured with OD280/OD260 ratio, which was next reverse transcribed into the cDNA through exploiting the cDNA reverse transcription kit with high capacity. Consequently, the Hepatitis B viral replication genes relative expression was identified through SYBR Green Master Mix following the treatment of this compound. We applied the $2^{-\Delta\Delta C_t}$ approach for the relative quantification of tripligate preformation.

3 CCK-8 assay

The CCK-8 assay was implemented to explore the novel compound's toxicity against the normal human liver cells. This preformation was performed following the instructions with slight modification. In brief, the human normal liver cells isolated from the normal animal were seeded into 96-well plates with 10^5 cells each well concentration. After that, the cells were cultivated in a 37°C incubator with 5 percent of CO₂ for half a day. As the cell enters the phase of logical growth, the compound was added with different concentrations for treatment. After the treatment, the culture medium of cell was discarded and the fresh culture medium involving reagent of CCK-8 was added into cells to implement the in-depth incubation. Eventually, for each well, its absorbance was tested at 490 nm. This research was accomplished at least 3 times, and the results were expressed with mean $\pm$ SD.

Ethical approval: The conducted research is not related to either human or animal use.

4 Results and discussion

4.1 Crystal structure of compound 1

The study of X-ray diffraction indicated that compound **1** was crystallized in an orthorhombic space group of *Pnma*, and it reflected a 3-dimensional porous structure. Its fundamental unit contains 0.5 Cd(II) ion, 0.5 bci³⁻, 0.5 free hydronium ion, and a lattice molecule of water. As displayed in the Figure 1a, Cd1 is situated in a triangular prism and is 6-coordinated through five oxygen atoms (namely, O1a and O2a, O1b, O2b, and O4c) and 1 N atom (N2) from four divergent bci³⁻ ligands, and the separations of Cd-O are between 2.243(3) and 2.526(4) Å, and the spacing of Cd-N is 2.186(5) Å. The bci³⁻ utilities as a μ_4 -bridge involve four divergent Cd(II) ions and its two carboxylate groups in chelating mode, one ketone oxygen atom, and one nitrogen atom both in monodentate mode (Figure 1b). Based on previous reports, such unique bridge mode of the bci³⁻ ligand has not been reported. All of the Cd(II) are bridged via bci³⁻ into a 3-dimensional anionic framework (Figure 1c). Notably, no cation could be defined from the different Fourier maps; thus, we can hypothesize that one lattice water molecule was protonated into a hydronium ion to balance the charge of the 3D anionic framework. The crystallographic *a* axis shows 1D rhombic channels. The solvent-accessible quantity of **1** counted through the PLATON program is approximately 26.0% per unit cell. In topology, bci³⁻ and Cd(II) ions could be considered as four-linked nodes; therefore, the whole 3D framework of compound **1** could be shortened into a four-linked **sra** topological network that has {4²·6³·8} point symbol (Figure 1d).

4.2 PXRD pattern and TGA

We characterized the as-synthesized bulk specimens through powder X-ray diffraction analysis to demonstrate the phase purity. The result is shown in Figure S1. Evidently, the diffraction summits of the experimental mode fit smoothly with that of the simulated mode based on the single-crystal diffraction information, suggesting that it contains good phase purity.

The thermal behavior of **1** was investigated via TGA, which was executed on the polycrystalline specimens in the nitrogen atmosphere. In the **1**'s curve of TGA (Figure 2), the release of the lattice water molecules occurred between

Figure 1: (a) Coordination surroundings of Cd(II) ion in compound **1**. (b) The coordination mode of the bci^{3-} ligand. (c) The 3-dimensional porous skeleton of complex **1** was observed along the crystallographic *a* direction. (d) Schematic diagram of the four-linked sra topological network for compound **1**.

85 and 106°C (obsd: 12.22%, calcd: 12.37%), and then, no weight loss occurred until 285°C, from which the skeleton of **1** began to decompose. The remaining weight of 29.48% corresponded to the formation of CdO (calcd: 29.41%).

4.3 Photoluminescent property of **1**

The luminescent spectra of H_3bci and compound **1** have been studied at normal temperature and pressure. As shown in Figure 3a, the H_3bci ligand shows a 348 nm emission band when excited by 320 nm, and **1** performs a 366 nm emission band (λ_{ex} is equal to 330 nm) similar to the free H_3bci ligand. The central Cd(II) ion is hard to be reduced or oxidized owing to its electronic configuration of d^{10} ; as a result, the complex **1**'s luminescence may be originated from the intraligand charge transfer. In contrast to the emission of H_3bci , the complex **1**'s redshift emission of 18 nm might result in the bci^{3-} coordination to the metal ions [21].

The **1**'s porous structure and strong emission in-depth inspired us to explore its sensing characteristics for determining metal ions. In evaluating its sensing

abilities for divergent metal ions, 2 mg of finely ground specimens of **1** were separately disseminated into 2 mL of $\text{M}(\text{NO}_3)_n$ in water (0.01 mol/L, $\text{M} = \text{Al}^{3+}$, Pb^{2+} , Co^{2+} , Hg^{2+} , Na^+ , Cu^{2+} , Ni^{2+} , Ag^+ , and Fe^{3+}), which were ultrasonically treated for 30 min, and then we measured the luminescent spectra of the above-mentioned suspension. In addition, the blank test without metal ions was conducted at

Figure 2: TGA curve of **1**.

Figure 3: (a) Emission spectrum of the H_3bci ligand and compound **1**. (b) The luminescence intensities of compound **1** disseminated in water and divergent metal ion solutions. (c) The complex **1**'s emission spectra disseminated in Fe^{3+} in water with diverse concentrations. (d) SV plots of I_0/I versus different concentrations of Fe^{3+} ions for **1**.

identical conditions. As shown in Figure 3b, in contrast to the result of the blank test, the luminescence intensity of **1** is greatly decided by the metal ions identities. In particular, Fe^{3+} ions can completely quench the luminescence of **1**. Titration experiments of Fe^{3+} were also performed by gradually adding Fe^{3+} concentration to evaluate its sensitivity. As reflected in Figure 3c, the **1**'s luminescence strength was progressively undermined with increasing concentration of Fe^{3+} . When the Fe^{3+} concentration reached 0.80 mM, **1**'s luminescence was nearly totally quenched. The Fe^{3+} -induced quenching function of **1** could be calculated using $I_0/I = A \exp(R[M]) + b$, an exponential quenching equation in which I and I_0 are the luminescent strength with or without Fe^{3+} ; $[M]$ represents Fe^{3+} concentration (mM); b , R , and A are the constants. The fitting result is as follows: $I_0/I = 0.8739 \exp(3.77 \times 10^{-3}[M]) + 0.5737$ (Figure 3d). In the low concentration range of 0–0.16 mM, the Stern–Volmer (SV) plot follows a linear relationship; with $I_0/I = 1 + K_{sv}[M]$, the SV equation, we can calculate quenching constant (K_{sv}), which is $1.136 \times 10^4/M$. Moreover, the Fe^{3+} detection limit

was calculated by $3\sigma/K_{sv}$, obtaining a low detection limit value of $2.15 \times 10^{-4} M$. These results indicated that **1** had high sensitivity toward Fe^{3+} ions.

4.4 Compound significantly reduced the level of TNF- α released into the plasma in a dose-dependent manner

After the synthesis of the new compound with novel structure, its treatment of Hepatitis B was determined. During the procession of the Hepatitis B infection, there was usually combined with an increased level of inflammatory cytokines. Thus, in the current study, we performed ELISA detection to detect the content of TNF- α released into the plasma after compound treatment. As the results shown in Figure 4, we can see there was a remarkably enhanced level of TNF- α in the plasma of model group, which is significantly higher than the levels in control group. There was a significant difference

Figure 4: TNF- α content in the plasma determined with ELISA assay. The Hepatitis B virus was used to infect the animal for the construction of the Hepatitis B animal model ($n = 10$). The mixture compound was then utilized for therapy at the concentration of 1, 2, and 5 mg/kg. ELISA detection was performed and the content of TNF- α released into the plasma was measured 6 and 12 h after compound therapy. ns means $P > 0.05$, * means $P < 0.05$, ** means $P < 0.01$, and *** means $P < 0.005$. The experiment was repeated at least three times, and the results were presented as mean $\pm$ SD.

between these two groups, with $P < 0.005$. After the compound treatment, the level of TNF- α released into the plasma was decreased. The inhibition of the new compound exhibited a dose-dependent manner.

4.5 Compound inhibited the relative expression of Hepatitis B viral replication gene

In the above research, the mixture can remarkably decrease the level of TNF- α liberated into the plasma in a dose-

dependent manner, suggesting the excellent application values of the new mixture on the Hepatitis B animal model treatment through inhibiting the inflammatory response. Nevertheless, whether this compound could also influence the replication of the Hepatitis B virus still need to be explored. Therefore, in this experiment, real-time RT-PCR was implemented to determine the relative expression of the Hepatitis B viral replication gene. The information in Figure 5 suggested that compared with the higher level of Hepatitis B viral replication gene in the control group, the mixture evidently downregulate the Hepatitis B viral replication gene relative expression levels with a dose-dependent pattern, which was in accordance with the previous

Figure 5: Expression levels of cccDNA measured by real-time RT-PCR. The compound was employed to be incubated with Hepatitis B at 10, 20, and 50 ng/mL concentration ($n = 5$). Real-time RT-PCR was performed to measure the Hepatitis B viral replication gene relative expression levels under compound incubation for 6 and 12 h. * means $P < 0.05$, ** means $P < 0.01$, and *** means $P < 0.005$. The experiment was repeated at least three times, and the results were presented as mean $\pm$ SD.

Figure 6: Viability of the normal human liver cells detected by CCK-8 assay. The human normal liver cells were treated via the fresh compound at serial concentrations (1, 2, 4, 8, 10, 20, 40, and 80 μM), and then the liver cells viability was measured by the CCK-8 assay, the cells without compound treatment were used as control. The experiment was repeated at least three times, and the results were presented as mean $\pm$ SD.

results. All the results above indicated the mixture has good biological activity on the Hepatitis B therapy.

4.6 Compound exhibited no toxicity on human normal liver cells

We have demonstrated the novel compound's outstanding treatment activity against the treatment of Hepatitis B animal model through inhibiting Hepatitis B viral replication. Nevertheless, in the process of the treatment, the compound's toxicity against human normal liver cells still required to be investigated. As a result, in this research, the CCK-8 assay was employed for determination. The information is displayed in Figure 6. In contrast to control group, the fresh compound possessed nearly no effect against the normal human liver cells viability. Besides, no remarkable difference was observed between these two groups. This research suggested that the compound had outstanding biological activity against the treatment of Hepatitis B animal model without toxicity.

5 Conclusion

In this present research, a novel Cd(II) MOF was designed and synthesized in the hydrothermal condition, and this MOF shows strong luminescence at normal temperature, which could be used as the fluorescent sensor for the Fe^{3+} ion. The ELISA assay shows that the mixture can remarkably

reduce the release of TNF- α into the plasma with a dose-dependent fashion. Moreover, the Hepatitis B viral replication gene relative expression levels were inhibited by the new mixture with a dose-dependent pattern. Finally, the prepared Cd-complex showed potential application as a candidate drug for treating the Hepatitis B through inhibiting Hepatitis B viral replication, which could significantly reduce Hepatitis B viral replication without toxicity.

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Data availability statement: The complex 1's selected bond angles ($^\circ$) and lengths ($\text{\AA}$); the patterns of PXRD of compound 1, the information could be observed in the supporting information file.

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