A New Zn(II)-containing Coordination Polymer for Photocatalytic Degradation of Organic Dyes and Treatment Activity on Atherosclerosis via Reducing the Vcam-1 Expression

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Abstract: By employment of a rigid tripodal nitrogen-containing heterotopic ligand tris(1-imidazolyl) benzene (Htib), a new fluorescent Zn(II)-containing coordination polymer \([\{\text{Zn(tib)}_2(\text{NO}_3)_2(\text{H}_2\text{O})_3\}\_n\) \(1\) with a rare two-fold interpenetrating \((3,6)\)-connected pyr network topology has been successfully prepared under the solvothermal reaction conditions. Under the condition of visible light irradiation, rhodamine B (RhB) and methylene blue (MB) could be degraded with good performance. In the biological function study, the cytotoxicity of the synthetic was evaluated with CCK-8 detection kit on human umbilical vein endothelial cells (HUVEC). The inhibitory effect of compound on vcam-1 expression in the vascular endothelial cells was evaluated by RT-PCR. The effect of the complex on the inflammatory response in the vascular endothelial cells was determined via ELISA test of IL-1β and TNF-α. The results of pose scoring software as well as molecular docking was conducted to explore the interaction between compounds and VCAM, which might provide latent regulatory mechanisms along with binding sites for compounds.

Key words: coordination polymer, photocatalytic degradation, vascular endothelial, molecular docking

1 Introduction

Coordination polymers (CPs) formed by organic bridging ligands as well as transition metal ions generate more and more interest in recent years, not only their broad applications prospects in material science field, but also their excellent performance and latent applications in electrochemistry, magnetism, catalysis, photochemistry along with biochemistry, and their attractive topologies as well as structures8-10. Organic dyes are one of the major pollutants in wastewater, the treatment of wastewater is a significant problem11. Since the photocatalytic degradation of phenol with MOF-5 was first reported, photocatalytic degradation of phenol via organic dyes based on CPs under visible light or ultraviolet has been rapidly developed because of its low cost as well as high efficiencies7,8. However, the number of CPs-based photocatalysts used for visible light irradiation is relatively small, because many CPs usually have low stability water12. On the other hand, rigid nitrogen-containing tripodal ligands are general ligands which have been utilized to construct metal-organic cage of attractive properties as well as structures or various porous CPs. The most commonly utilized nitrogen-containing donor groups, containing imidazole, triazole, pyridyl group, tetrazole as well as benzimidazole are attached to rigid benzene ring, forming a number of rigid nitrogen-containing tripodal ligands13-15. Recent literatures have revealed that the imidazole-based ligands are good candidates for building coordination polymers with excellent water stabilities, and some of them have already used in the photocatalytic degradation or adsorption of organic dyes16-19. For instance, Zhang’s group has reported that Co-based CPs assembled using 1,3,5-tris (1-imidazolyl) benzene ligands show good dyes degradation capacity. In this study, by employment of a rigid tripodal nitrogen-containing heterotopic ligand tris (1-imidazolyl) benzene (Htib), a new fluorescent Zn(II)-containing coordination polymer \([\{\text{Zn(tib)}_2(\text{NO}_3)_2(\text{H}_2\text{O})_3\}\_n\) \(1\) with a rare two-folded interpenetrating \((3,6)\)-connected pyr network topology has been successfully prepared under the solvothermal reaction conditions. The prepared complex 1 was completely measured by single crystal X-ray diffraction as well as elemental analysis. Under the condition of visible light irradiation, rhodamine B (RhB) and
methylen blue (MB) have fine photocatalytic degradation performance. We proposed the photocatalytic mechanism, and then confirmed it. In biological research, the protective effect of compound on atherosclerosis was evaluated. Firstly, the CCK-8 results indicated the compound has no cytotoxicity on HUVEC, and the RT-PCR detection of vcam-1 in vascular endothelial cells indicated that this important adhesion molecule in progression of AS could be significantly reduced by compound treatment. Then, the ELISA detection of TNF-α and IL-1β revealed that the inflammatory level in the vascular endothelial cells was also been inhibited via compound. By calculating pose and docking scores, the latent binding patterns between target proteins and synthetic compounds were discussed.

2 Experimental

2.1 Chemicals and measurements

All the reagents as well as solvents are analytical grade and can be applied without deep purification. Using the Perkin-Elmer 240C elemental analyzer to obtain elemental contents of C, N and H element content. With Avatar 360 (Nicolet) infrared spectrometer we collected FT-IR spectra (KBr pellets) in the region of 4000-400 cm⁻¹. Utilizing Shimadzu UV-3101PC instrument we carried out ultraviolet–visible spectra. Applying the Edinburgh Instrument FL/FS-920 fluorescent spectrometer we recorded fluorescent spectra under room temperature.

2.2 Preparation and characterization for [Zn(tib)₂(NO₃)₂·6H₂O]

We added Zn(NO₃)₂·6H₂O which is 0.3 mmol and 0.0409 g and tib of 0.3 mmol and 0.0828 g, mixed them to form a mixture, sealed it into a Teflon-lined stainless steel Parr bomb of 25 mL which has 10 mL deionized H₂O, heated it for 3 days at 140°C, and then cooled it to room temperature. We acquired a small white crystals of 1 with block-like (the yield is 72% on the basis of Zn(NO₃)₂·6H₂O), and then washed by using deionized ethanol and water. Anal. Calc for Anal. Calc for C₃₀H₂₄N₁₂ZnO₁₂: C, 46.31; H, 3.63; N, 25.20%; found: C, 46.69; H, 3.43; N, 25.55%. IR (KBr, cm⁻¹): 3385 (m), 2360 (w), 1944 (w), 1829 (w), 1610 (m), 1583 (m), 1446 (m), 1398 (w), 1315 (w), 1186 (w), 1142 (w), 1109 (w), 1051 (w), 1014 (w), 922 (w), 905 (w), 862 (w), 810 (w), 779 (m), 746 (w), 709 (w), 670 (w), 635 (w), 592 (w), 526 (w), 477 (w), 426 (w).

By using Oxford Xcalibur E diffractometer we acquired X-ray data of compound 1. Utilizing CrysAlisPro software for the sake of analyzing intensity data and convert them into HKL files. The original structural models of complexes 1 was constructed through SHELXS program according to the direct method and corrected by utilizing SHELXL-2014 program based on the least-squares method. Mixing all the non-H atoms of complex 1 which have anisotropic parameters, and the whole H atoms were fixed to their connected C atoms geometrically through applying AFIX commands. Table 1 describes the numerical information as well as crystallographic parameters reorganized of compound 1.

2.3 Cell Counting kit-8 detection

The cytotoxicity of the compound was evaluated on HUVEC with CCK-8 detection kit under the guidance of the instructions with some modification. In brief, the HUVEC cells in the logarithmic growth phase were collected and seeded into 6-well plates at the concentration of 5 × 10⁴ cells/well, and the cells were cultured in an incubator at the condition of 37°C, 5% CO₂ humidified atmosphere. When the cells reach confluence of 70-80%, serious dilutions of the compound (1, 2, 4, 8, 10, 20, 40, 80, 100 μM) were added into wells for 24 h incubation. After that, the cells were harvested and centrifuged at 800 rpm for 5 min. The culture medium was discarded, and the 10% CCK-8 (Dojindo Laboratories, Kumamoto, Japan) in 100 μL medium without FBS was added into wells for 2 h incubation at 37°C in the dark. Subsequently, the absorbance of each well was measured with Thermo Scientific Microplate Reader at 450 nm. The Cell viability curves were calculated.

Table 1 Crystallographic parameters and refinement details of complex 1.

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>C₃₀H₂₄N₁₂Zn</th>
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<tr>
<td>Final R indexes [I&gt; = 2σ (I)]</td>
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<tr>
<td>Final R indexes [all data]</td>
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and plotted according to the absorbance values. Three replicate wells were used to determine each point.

2.4 RT-PCR detection of vcam-1

15 New Zealand rabbits were used in this experiment, the rabbits were randomly divided into three different groups \((n=5)\). The control group (received sham-operation), model group (received endothelium injury without treatment) and the treatment group (received endothelium injury with compound treatment). After the construction of endothelium injury model, the compound was given daily for 14 days treatment. At the end of this treatment, the carotid artery segments were harvested for the detection of relative expression of vcam-1 with RT-PCR preformation. This experiment was performed according to the protocols precious introduced with some modification. The primer used in this experiment were vcam-1: GGACCA-CATCTAGCTGACA and TTGACTGTGATGGCTTCCC, gapdh: AATGGGCAAGCGTATAGGAAA and GC-GCCAATACGACAAATC.

2.5 ELISA detection of IL-1β and TNF-α

After the endothelium injury model construction and compound treatment, the level of inflammatory response in the animals as evaluated by the ELISA measurement of TNF-α and IL-1β in the endothelium tissues. In brief, at the end of this treatment, the carotid artery segments were harvested, grinded and suspended in PBS, the content of TNF-α and IL-1β was measured via ELISA detection kit following the instructions. This experiment was repeated at least three time. The results were presented as mean ± SD.

2.6 Molecular Docking

Discovery Studio 3.0 serves as a commercial software that can predict latent binding pattern between protein and small molecule, thus, it was selected a platform for carrying out molecular docking simulation. Its advantage is that it has been customized at the aim of better developing high-precision scoring function. We derived ligand structure from the crystal structure measured via X-ray, and from protein date bank (PDB) we downloaded it. The VCAM protein crystal structure (PDB ID: 1VSC) was utilized to predict the docking, and the coordination posture was scored for the high resolution. The gesture scoring module as well as Libdock tool were utilized for preparing the structures of receptor and ligand for molecular docking simulation. The grid frame length is set at 40, which is enough large to cover the whole docking bag, including double helix chains along with protein parts.

3 Results and Discussion

3.1 Molecular structures

The hydrothermal reaction is a fine choice for preparing coordination polymers which have excellent water stability. Such a reaction condition usually involves reaction of the starting materials in a highly sealed reaction container using the pure water as the solvents. High temperature and high pressures accompanies with the reaction process. Complex 1 chemical formula was established by elemental analysis as well as single crystal X-ray diffraction, the formula was \([\text{Zn(tib)}_2\text{(NO}_3)_2\text{(H}_2\text{O})_2]\) \(_\alpha\). Complex 1 could not be dissolved in the common solvents such as water, DMF, EtOH and so on. The refinement results as well as structural solution based on crystal data collected at room temperature reflect that cubic space group la-3, and presents a three-dimensional porous framework. According to Fig. 1a, 1’s asymmetric unit contains one sixth of a crystallographically independent Zn(II) ion, one third of tib ligand, as well as one third of NO\(_3\) ion. Every Zn(II) is surrounded via six nitrogen atoms, formed by two six imidazolyl nitrogen atoms in six different tib ligands. We can described the coordination geometry which around the center of Zn(II) as a warped octahedron. The Zn-N bond distance is 2.182 Å, which belongs to the normal range values reported in other Zn(II)-based coordination polymers based on the N-donor ligands\(^{17-20}\). The tib ligand has C\(_3\) axis through the center of benzene ring, this means that three imidazolyl groups cannot be distinguished during assembly (Fig. 1b).

The tib ligand, as an isoligand, binds to three randomly oriented Zn(II) ions. Each Zn(II) ion links six tib ligands, and each tib ligand links three Zn(II) ions, so combining Zn(II) ions as well as tib ligands cause a complex three-dimensional framework (Fig. 1c). At the aim of better understanding the relationship between tib ligands as well as Zn(II) ions, three-dimensional framework topological structure is analyzed. Simply put, the tib ligand is connected with three Zn(II) ions, which can be regarded as three connecting nodes, while Zn(II) ion is connected with six tib ligands, which can be regarded as six connecting nodes. Then, the obtained network is a pyr network connected by two peaks (3,6). Its Schlafli symbol is \([6^3\times12.8^4\times3]\) \(_\text{6^3}\), as shown in TOPOS analysis. Such a single network includes large gaps, so the two single networks penetrate into each other, forming a pyr framework of double mutual penetration (Fig. 1d).

To check the phase purity of the products, powder X-ray diffraction (PXRD) experiments have been carried out for complex 1. The peak positions of the experimental and simulated PXRD patterns are in good agreement with each other, indicating that the crystal structures are truly representative of the bulk crystal products (Fig. 2a). The differences in intensity may be owing to the preferred orientation of the crystal samples. The thermal stability of complex 1 was determined in the temperature range of
The thermal stability of 1 was investigated from the ambient temperature to 800°C under the N₂ atmosphere. The thermal decomposition of 1 occurs in three main stages with the structure remaining approximately unchanged up to ca. 400°C. Given that the first two losses overlap, they have been considered together as a unique loss in the range ca. 21–390°C. Overall, 1 loses in this range approximately 6.5% of the total weight, which agrees well with the release of three water molecules per formula unit (calculated value of 6.8%).

### 3.2 Photocatalytic degradation of organic dyes.

Free tib and 1’s solid-state luminescence were measured at room temperature. When title compound is excited at 328 nm, there is a strong emission peak at 394 nm (Fig. 3a). For comparison, when excited at 312 nanometers, free tib ligands reveals emission at 412 nanometers. 1’s strong emission is due to charge transfer in ligands. We determined crystal 1’s optical diffuse reflectance spectra under room temperature. Via extrapolating the linear part of absorption edge, 1’s energy band gap ($E_g$) was 2.30 eV, indicating its latent semiconductor properties (Fig. 3b).

The photocatalytic properties of photocatalysts were investigated using organic dyes Rhodamine B (rhB) along with methylene blue (MB) as dye models. The photocatalytic properties of 1 for MB degradation under the irradiation of visible light were studied. After 15, 30, 45 and 60 minutes of the irradiation of visible light, MB degradation efficiency under catalyst 1 was 33.2%, 56.3%, 73.6% and 86.2%, respectively (Fig. 3c). After 75 minutes, MB degradation
efficiency can up to 92.8%. Compared with above experiment, in the blank experiment which only H\(_2\)O\(_2\) MB degradation ratestructure were 16.4, 34.2 and 58.7%, respectively, for commercial inorganic oxide semiconductor titanium dioxide (20 nm) as well as titanium dioxide (60 nm) after the irradiation of visible-light for 75 minutes. 1 for MB degradation photocatalytic efficiencies were more than that of TiO\(_2\) (20 nm). TiO\(_2\) (20 nm) for MB degradation photocatalytic efficiencies were higher than that of TiO\(_2\). After 15, 30, 45, 60, 75, 90 and 105 minutes irradiation of visible light, RhB under catalyst 1 degradation efficiencies are 18.8, 36.5, 52.7, 64.9, 73.1, 80.2 and 85.2%, respectively (Fig. 3d). After 2 hours, RhB degradation efficiency can up to 90.6%. Compared with above experiment, in the blank experiment which only H\(_2\)O\(_2\), RhB degradation efficiencies were 2.8, 16.3 as well as 41.5%, respectively, for commercial inorganic oxide semiconductor titanium dioxide (20 nm) as well as titanium dioxide (60 nm) after the irradiation of visible light for 2 hours. 1 for RhB degradation photocatalytic efficiencies were more than that of TiO\(_2\) (20 nm). TiO\(_2\) (20 nm) for RhB degradation photocatalytic efficiencies were higher than that of TiO\(_2\) (60 nm). Thus, 1 is a fine photocatalyst for degrading RhB as well as MB. At the aim of understanding RHB and MB photocatalytic degradation mechanism under the action of catalyst (1), photocatalytic experiments were carried out under ammonium oxalate (AO, h\(^+\) scavenger), mannitol (OH scavenger), and benzoquinone (BQ, O\(^2^-\) scavenger) as well as other various scavengers. Under AO, mannitol and BQ, MB degradation efficiency reduced from 92.8% for 1 to 80.7, 54.3 and 23.2%, respectively. Experimental results reveal that h\(^+\) was the major active substance, 'OH radical and O\(^2^-\) promoted the degradation of MB to some extent. Under AO, mannitol and BQ, MB, RhB degradation efficiencies reduced from 90.4% for 1 to 58.7, 11.4 and 3.2%, respectively. Experiment results reveal that h\(^+\) and the 'OH radical were major active substance, and O\(^2^-\) promoted the degradation of RhB to some extent.

The reaction kinetics model has an important role in the development of a photocatalytic degradation process for industrial application, because it can explain the effects of the necessary parameters on the photodegradation reaction rate, independently of the shape and configuration of the reactor. The Langmuir–Hinshelwood (L–H) kinetic model has been used for the photodegradation of many organic compounds such as dyes. This model is widely accepted to explain the kinetics of photodegradation processes. In(C\(_0\)/C\(_t\)) versus time was plotted for photodegradation of Rhb using 1 (Fig. 4). The experimental data fit well, with R\(^2\)>0.99, which indicates that the experimental data can be described by the pseudo-first order kinetics model. The slope of this linear plot indicates the first order rate constant of 1 is 0.12 min\(^{-1}\), which is larger than that of some CP-based materials under the similar conditions\(^{21-23}\).
3.3 Cytotoxicity of the compound on HUVEC

CCK-8 detection kit was performed to detect the cytotoxicity of the synthetic compound on HUVEC. The HUVEC were treated with serially diluted compound for 24 h, with Oxaliplatin was used as the positive control drug. The absorbance values at 570 nm was measured, which reflect the cancer cells viability after compound treatment. As the CCK-8 results shown in Fig. 5, the compound did not show any inhibitory effect on the viability of the HUVEC cells, while the positive drug could reduce the viability of the cells to about 18% after 24 h treatment. This result indicated that the compound has no cytotoxicity on HUVEC, which was recommended for the following experiment.

3.4 Compound reduced the relative expression level of vcam-1

VCAM-1 has a significant function in AS course, which involved in the adhesion of cells, cells and extracellular matrix components. Recent reports revealed that the content of VCAM-1 in the vascular cell determines the consequence of the AS development. Thus, in this experiment, we firstly constructed the animal model and then evaluated the effect of compound on vcam-1 relative expression in the intravascular cells. As the results showed in the Fig. 6, at the 7th and 14th day after compound treatment, the expression of vcam-1 in the model group was significant increased compared with the control group, however, this up-regulated expression could be obviously reduced by the compound treatment to about the normal level.

3.5 Compound reduced the content of IL-1β and TNF-α

The various factors leading AS will ultimately cause the pathological processes of chronic inflammation, reflected as increased level of inflammatory cytokines TNF-α and IL-1β. So, in this study, the inhibitory effect of compound in reducing the level of inflammatory response in intravas-
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Fig. 6 Reduced relative expression level of \textit{vcam-1} in intravascular cells after compound treatment. After the model construction and compound treatment, the carotid artery segments were harvested for the detection of relative expression of \textit{vcam-1} with RT-PCR.

Fig. 7 Reduced TNF-\(\alpha\) and IL-1\(\beta\) level in intravascular cells after compound treatment. After the model construction and compound treatment, the carotid artery segments were harvested, and the TNF-\(\alpha\) and IL-1\(\beta\) level was detected via ELISA test.

cular cells was further examined with ELISA assay. According to the results revealed in Fig. 7, the content of TNF-\(\alpha\) and IL-1\(\beta\) in model group was obvious higher than that in control group \((p < 0.005)\), and the compound treatment could significantly reduce the level of TNF-\(\alpha\) and IL-1\(\beta\) to almost the normal level.

3.6 Molecular Docking
These results indicated that VCAM proteins have latent binding affinity with synthesized compounds, but their latent interaction modes are still missing. To further understanding possible binding patterns between VCAM protein and synthesized compound, pose scoring as well as molecular docking was carried out. Figure 8A exhibits the whole view of the possible binding pattern, where we can observe that the yellow rod of the compound indicates that it has been processed in the docking bag. From the surface view, the multiple functional groups of the compound showed potentials of interaction with VCAM. The surface binding view shown in Fig. 8B clearly reveals the binding interaction between receptors and ligands. It can be observed that the functional groups of this compound showed latent binding ability on the binding surface between predicted binding site and helix chain, which may affect the efficacy of VCAM. The compound interacts with mono receptor of VCAM protein, forming polar contacts with Glu96, His98 and Ser100. Other than other residues, hydrophobic interactions are reinforced by Leu124, Ile126, Gly131 and Leu134. The compound obtains a docking score as 78.5364, which reveals a moderate binding ability.

4 Conclusion
In conclusion, we have made a luminescent Zn(II)-based coordination polymer by using a rigid tripodal nitrogen-containing heterotopic ligand tris(1-imidazolyl) benzene (\textit{Htib}). The prepared complex 1 was completely measured.
by single crystal X-ray diffraction as well as elemental analysis. Under the condition of the irradiation of visible light, rhodamine B (Rhb) and methylene blue (MB) could be degraded with a good performance. In biological study, we aimed to explore new candidates for the treatment of atherosclerosis. The CCK-8 results indicated the compound has no cytotoxicity on HUVEC. Next, the protective effect of compound was evaluated in the constructed animal model. The RT-PCR determination of vcam-1 in vascular endothelial cells indicated that the compound could significantly inhibit the expression of vcam-1. Besides, the ELISA detection of TNF-α and IL-1β revealed that the inflammatory level in the vascular endothelial cells was also been inhibited by compound. The results from molecular docking provided latent binding patterns between targeted protein (VCAM) and synthesized compound, indicating the possible binding mode and regulation mechanism for the compound.

Fig. 8 Interaction of the compound and VCAM protein. (A) A whole view of binding pattern of the compound interacting with receptor protein VCAM (PDB: 1VSC). Proteins are banded as well as compounds are yellow rods. (B) Surface binding pattern local views exhibit that the compound indicates latent binding affinity with VCAM by a direct binding with its functional groups.

Reference
11) Zhang, J.P.; Horike, S.; Kitagawa, S. A flexible porous coordination polymer functionalized by unsaturated