Research Article

Two Mixed-Ligand Cu(II) Polymers: Treatment Activity on Acute Cerebral Infarction Combined with Alteplase

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1. Introduction

The etiological basis of cerebral thrombosis is mainly atherosclerosis, so the factors that produce atherosclerosis are the most common causes of cerebral infarction. The results of the recent INTERSTROKE study conducted worldwide show that 90% of the risk of cerebral infarction can be attributed to 10 simple risk factors, which are high blood pressure, smoking, excessive waist-to-hip ratio, improper diet, lack of physical activity, diabetes, excessive alcohol consumption, excessive mental stress and depression, underlying heart disease, and hyperlipidemia [1, 2]. A large number of clinical practices have proved that the early implementation of intravenous thrombolysis after cerebral infarction can promote the recovery of local blood supply as soon as possible and improve the abnormal neurological function.

In the past several years, the researches on producing the CPs from organic ligands and transition metals have been widespread developed because of their diversity in crystallography along with the underlying applications in biomedicine and molecular recognition as well as catalysis [3–5]. The CPs’ ultimate skeletons are influenced via distinct factors, containing the temperature of the reaction; the shapes, size, coordination positions, and flexibility of the chose organic ligands; the coordination patterns of center metal; and the synthetic strategy, ratio, and volume of solvents, together with chemical polarity [6–9]. At present, most studies have paid attention to the O- or N-donor ligand, and the mixed-ligand method of combining the O- or N-donor ligands in a reaction system has been demonstrated to be a feasible method to produce the CPs containing fascinating performances and novel architectures [10–14]. In this study, with the reaction between Cu(NO3)2·3H2O and 4-(2′,3′-dicarboxylphenoxy) benzoic acid (H3L) in the presence of N-donor co-ligand 1,2-bis(4-pyridyl) ethylene (bpe), two novel mixed-ligand coordination polymers (CPs), i.e., [Cu(H2L)2(bpe)(H2O)2]·(H2O)4 (1) and [Cu3(L)2(bpe)3(H2O)4]·(H2O)6 (2), have been created under different solvent systems. Furthermore, the biological activity of compounds was evaluated, and the related mechanism was explored as well. Firstly, the enzyme-linked immunosorbent assay (ELISA) was conducted to measure the content of coagulation factors in the serum after compound treatment. In addition to this, the real-time reverse transcription-polymerase chain reaction (RT-PCR) assay was conducted, and the inflammatory response in the brain was also determined.
coordination polymers are structurally tested via utilizing the diffraction of single-crystal X-ray, which were in-depth characterized employing TGA, PXRD, and EA. The ELISA assay and real-time RT-PCR were conducted, and the treatment of compounds was measured.

2. Experimental

2.1. Chemicals and Measurements. All of the reactants exploited in this research with reagent grade were purchased from market source and utilized with no further modifications. And the generations were carried out in stainless steel autoclaves lining by Teflon (20 mL) under an autogenous pressure. A Perkin-Elmer 240C was employed for conducting the elemental analysis. Thermal analysis was implemented with the thermogravimetric analyzer of ZRY-2P with 10°C/min heating rate under the air flow between 30 and 700°C. IR spectra could be gathered from 400 to 4000 cm⁻¹ through employing Infrared spectrometer of Nicolet Avatar-360.

2.2. Preparation and Characterization for [Cu(H₂L)₂(bpe)(H₂O)₂]bpe·(H₂O)₆ (1) and [Cu₂(H₂L)₂(bpe)(H₂O)₆]·(H₂O)₆ (2). The mixture formed by 18 mg and 0.1 mmol of bpe, 0.1 mmol and 30 mg of H₂L, 36 mg and 0.15 of mmol Cu(NO₃)₂·3H₂O, and 5.0 mL of distilled water (the pH value was adjusted to 5 with HCl) was stored in a stainless steel vessel that lined by Teflon (20 mL), which was subsequently heated under a temperature of 120°C for three days. After cooling it slowly to RT, the compound’s blue massive crystals were gathered with approximately 23.0% yield in accordance with H₂L. Anal. Calcd for C₆₆H₆₄N₆O₂₄Cu₃: N, 4.92%, C, 56.97% and H, 4.43%. Found: N, 4.85%, C, 57.02% and H, 4.58%. IR (KBr, cm⁻¹, Figure S1): 551(m), 689(w), 764(m), 824(m), 1021(w), 1158 (w), 1243 (m), 1395(m), 1454(w), 1558(s), 2362(m), 3049(m), 3364(m).

The mixture formed by 18 mg and 0.1 mmol of bpe, 0.1 mmol and 30 mg of H₂L, 36 mg and 0.15 of mmol Cu(NO₃)₂·3H₂O, and 5.0 mL of DMF was maintained in a stainless steel vessel that lined by Teflon (20 mL), which was subsequently heated under a temperature of 120°C for three days. After cooling it naturally to RT, the compound’s blue massive crystals were gathered in approximately 36.0% yield in the light of H₂L. Anal. Calcd for C₅₄H₄₀N₄O₂₄Cu₃·2H₂O (2): N, 5.54%, C, 52.29% and H, 4.26%. Found: N, 5.49%, C, 52.68% and H, 4.35%. IR (KBr, cm⁻¹, Figure S1): 555(m), 785(m), 833(m), 1071(w), 1102(w), 1165(w), 1243 (m), 1301(w), 1388(s), 1473(w), 1533(s), 1612(s), 2362(m), 3049(m), 3364(m).

The SuperNova was utilized for acquiring the data of X-ray. And the CrysAlisPro was employed for analyzing the intense data, which was next converted next to the HKL files. The SHELXS in the light of direct mean together with the SHELXL-2014 software according to least squares strategy were employed, respectively, for the synthesis and refinement of original architectural modes. After the use of entire nonhydrogen atoms, we mixed anisotropic parameters. Ultimately, the whole H atoms were next fixed on the C atoms, which are bridged with AFIX commands in the geometry. The as-prepared compounds’ refinement details as well as their crystallography parameters are displayed in Table 1.

2.3. ELISA Assay. After the synthesis of compounds 1 and 2 with novel structures, their treatment activity the on acute cerebral infarction was assessed, and the related mechanism was explored as well. In brief, 50 mice (5-6 weeks, 20-22 g) were used in this research. All the mice were kept at the standard condition of 20–25°C, 45% humidity for 3 days before the experiment. After the animal is anesthetized, the acute cerebral infarction animal model was induced. Then, compounds 1 and 2 were injected for treatment at the concentration of 5 mg/kg. After that, the serum was collected, and the content of coagulation factors in the serum was measured. This research was repeated at least three times, and the results were presented as mean ± SD.

2.4. Real-Time RT-PCR Assay. To determine the inhibitory activity of the new compounds on the inflammatory response in the brain, the real-time RT-PCR was conducted in this research. This preformation was conducted totally under the guidance of the instructions with only a little change. In brief, 50 mice (5-6 weeks, 20-22 g) were used in this research. All the mice were kept at the standard condition of 20–25°C, 45% humidity for 3 days before the experiment. After the animal is anesthetized, the acute cerebral infarction animal model was induced. Then, compounds 1 and 2 were injected for treatment at the concentration of 5 mg/kg. After that, the brain tissue was collected, and the total RNA in the tissue was isolated. The concentration of the total RNA was measured and then reverse transcribed into the cDNA. Finally, the real-time RT-PCR was conducted, and the inflammatory
two carboxylic acid oxygen atoms originated from two L3 molecules (Figure 2(a)). Based on Figure 2(a), the Cu1 ion reveals two separate Cu2+ ions in crystallography (namely, Cu1 and Cu2); a L3 molecule; bpe I, bpe II, and bpe III ligands (with 0.5 site of occupancy); and the lattice O12, O11, and O10 molecules as well as the coordinated O8 and O9 molecules (where the Cu1-O2, Cu1-O8, and Cu1-N1 distances of 2.043(7), 2.090(3), and 2.168(1) Å). The Cu2 ion exhibits the twisted structure of octahedron coordinated through three carboxylic acid O atoms derived from two L3 molecules (the distances of Cu2-O4, Cu2-O6a, and Cu2-O7a are 2.034(9) Å, 2.130(9) Å, and 2.127(7) Å) and two N atoms (the distances of Cu2-N2, Cu2-N3 are 2.093 Å and 2.052(6)) and a coordinated H2O molecule and bpe II, creating O⋯H⋯O and O⋯H⋯N H bonds with carboxylic acid O14, O12, and O11 and O10, O16, and O5 as well as O4 atoms and coordinated Ow1 and Ow2 molecules. The separations of O⋯O and N⋯O (2.568–3.049 Å and 2.597–2.610 Å, Figure S2), which are comparable with the separations, existed in reported complexes. All of the weak interactions make the 3-dimensional supramolecular net more stable. It is worth nothing that the H2L molecule possesses no practical contribution to the establishment of 1-dimensional chain architecture of the compound 1; it only saturates the Cu2+ center coordination.

And the single-crystal architecture analysis suggests that 2 exists in triclinic space group of P1; it displays a 3-dimensional skeleton. In its fundamental unit, there exist two separate Cu2+ ions in crystallography (namely, Cu1 and Cu2); a L3− ligand; bpe I, bpe II, and bpe III ligands (with 0.5 site of occupancy); and the lattice O12, O11, and O10 molecules as well as the coordinated O8 and O9 molecules (Figure 2(a)). Based on Figure 2(a), the Cu1 ion reveals the octahedral structure, which is coordinated through two carboxylic acid O atoms derived from two L3− and two N atoms offered via two bpe together with two coordinated H2O molecules (where the Cu1-O2, Cu1-O8, and Cu1-N1 distances of 2.043(7), 2.090(3), and 2.168 (1) Å). The Cu2 ion exhibits the twisted structure of octahedron coordinated through three carboxylic acid O atoms originated from two L3− (the distances of Cu2-O4, Cu2-O6a, and Cu2-O7a are 2.034(9) Å, 2.130(9) Å, and 2.127 (7) Å) and two N atoms belong to two molecules of bpe (where the lengths of Cu2-N2 and Cu2-N3 are 2.093 Å and 2.052(6)) and a coordinated

### Table 1: The as-prepared compounds’ refinements details along with their parameters of crystallography.

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response in the brain tissue was measured, with gapdh used as the internal gene.

### 3. Results and Discussion

#### 3.1. Crystal Structures

The exploration of single-crystal architecture study indicates that complex 1 exists in the triclinic space group of P1 and reveals a 1-dimensional chain. Its fundamental unit contains one separate Cu2+ ion, two molecules of H2L− (namely, H2L− I and H2L− II), a bpe I ligand and a lattice bpe II molecule, and the coordinated water (Ow1 and Ow2) molecules as well as four lattice molecules of water (namely, Ow6, Ow5, Ow4, and Ow3) (Figure 1(a)). As illustrated in Figure 1(a), the Cu2+ ion displays the structure of octahedron, where two carboxylic acid oxygen atoms originated from two H2L− (and the distances of Cu1−O2 and Cu1−O9 are 2.058(4) Å and 2.059(3) Å) and two N atoms come from two bpe (with the Cu1−N1 and Cu1−N2 lengths of 2.113(3) Å and 2.115(8) Å) taking over equatorial plane. Two coordinated H2O molecules occupy the axial positions (where the distances of Cu1·Ow1 and Cu·Ow2 are 2.082(3) Å and 2.100(7) Å). Around Cu2+ ions, the angles of the bond are between 87.83° and 178.85°. According to Figure 1(b), Cu2+ ions are connected via bpe I to produce a 1-dimensional chain, and the angle of N1−Cu1−N2 is 178.6°. The neighboring 1-dimensional chains are deeply linked through H bondings between coordinated Ow1 and Ow2 molecules and the consecutive carboxylate O3 and O10 atoms, producing an ultimate 3-dimensional supramolecular net as displayed in Figure 1(c). In addition, the open channels were occupied by lattice H2O molecules and reveals a 1-dimensional chain. Its fundamental unit, there exist two separate Cu2+ ions in crystallography (namely, Cu1 and Cu2); a L3− ligand; bpe I, bpe II, and bpe III ligands (with 0.5 site of occupancy); and the lattice O12, O11, and O10 molecules as well as the coordinated O8 and O9 molecules (Figure 2(a)). Based on Figure 2(a), the Cu1 ion reveals the octahedral structure, which is coordinated through two carboxylic acid O atoms derived from two L3− and two N atoms offered via two bpe together with two coordinated H2O molecules (where the Cu1−O2, Cu1−O8, and Cu1−N1 distances of 2.043(7), 2.090(3), and 2.168 (1) Å). The Cu2 ion exhibits the twisted structure of octahedron coordinated through three carboxylic acid O atoms originated from two L3− (the distances of Cu2−O4, Cu2−O6a, and Cu2−O7a are 2.034(9) Å, 2.130(9) Å, and 2.127 (7) Å) and two N atoms belong to two molecules of bpe (where the lengths of Cu2−N2 and Cu2−N3 are 2.093 Å and 2.052(6)) and a coordinated...
water (the separation of Cu2-O9 is 2.117(9) Å). The L3− contains three modes: μ3: (k1-k^0)-μ1-COO-, (k1-k^0)-μ1-COO-, and (k1-k^0)-μ1-COO-. (Figure 2(b)). Between two benzene rings, the dihedral angle and ether bond angle are, respectively, counted to be 76.35° and 119.28°, revealing that L3− molecule has a semirigid character in compound 2. Each pair of neighboring Cu2 ions is connected via four carboxylic acid groups of two L3− to produce a dinuclear Cu2(COO)4 SBU (Figure 2(c)). bpe III links two SBUs together to generate a 1-dimensional chain architecture along axis b, which is deeply propagated through the bpe II molecules for the formation of a 2-dimensional layer net (Figure 1(b)). The acquiring 2-dimensional layers are ultimately supported via the non-ignorable bpe I-Cu1 chains to produce the ultimate 1’s 3-dimensional skeleton (Figure 2(c)). Within such net, the lattice O10, O11, and O12 molecules create H bonds to the consecutive carboxylic O6, O5, O4, and O3 atom; the length of O⋯O is between 2.683 and 3.119 Å (Figure S3). All of the weak interactions further enhance the stability of the 3-dimensional skeleton.

In topology, each Cu2+ center and the L3− ligand is reduced as 3- and 4-linked nodes, respectively. As a result, the whole complex 2 could be reduced to a 3,4,4-linked net with (105·12) (4·10^5)2 (4·8^5)2 point (Schläfli) symbol (Figure 2(d)).

In the aim of exploring the products’ phase purity, the PXRD study for the as-prepared CPs was finished (Figure 3(a)). Between the PXRD pattern peak positions of the simulation and experiment, there exist a well accordance, and this result suggests that the crystal architectures is a real representation of massive crystal products. The strength differences are probably resulted from crystal samples preferred selection. Simultaneously, the CPs’ thermostability is investigated between 25 and 800°C in air, as revealed in Figure 3(b). The TG curve suggests that 15% of constant weightlessness (with the calculated value of 16.1%) appeared when the temperature less than 270°C; this explains the disappearance of a lattice bpe molecule. The second weightlessness, under a temperature of 200-303°C, is owing to the thermal decomposition of two coordinated and four lattice
Figure 2: (a) The coordination manner for the Cu ions. (b) The coordination mode for the ligand. (c) The 2’s 3-dimensional net. (d) The 3,4,4-linked net of compound 2.

Figure 3: (a) The 1’s pattern of PXRD. (b) and its TGA curve.
water molecules (the found and calculated values are 9% and 9.5%). The further increase of temperature leads to the removal of the two organic ligands. The mass of the ultimate residue is equivalent to the full combustion of the complex into the associated metal oxide (CuO: calcd, 6.6%, found, 6.5%).

The compound 2's TG curve indicated that 8% of constant weightlessness (with the calculated value of 7.2%) appeared when the temperature is less than 110 °C; this explains the disappearance of six lattice H₂O molecule. The second weightlessness in the temperature ranges from 110 to 192 °C, because of the thermal removal of four coordinated molecules of H₂O (with the found and calculated values of 5% and 4.8%). The further increase of temperature resulted in the removal of two organic ligands, and the quality of the ultimate residue is equivalent to the full combustion of the complex into the associated metal oxide (CuO: calcd, 14.8%, found, 14%).

3.2. Compound Significantly Reduce Content of Coagulation Factors in the Serum. Early implementation of intravenous

**Figure 4**: Significantly reduced content of coagulation factors in the serum after compound treatment. The cerebral infarction animal model was constructed and the compound was injected for treatment at the concentration of 5 mg/kg. The content of coagulation factors in the serum was firstly conducted to measure with ELISA assay.

**Figure 5**: Obviously inhibited inflammatory response in the brain after compound treatment. The cerebral infarction animal model was constructed and the compound was injected for treatment at the concentration of 5 mg/kg. The real-time RT-PCR assay was conducted, and the inflammatory response in the brain was measured.
thrombolysis after cerebral infarction can promote the recovery of local blood supply as soon as possible and improve the abnormal neurological function. Thus, in this research, the content of coagulation factors in the serum was firstly conducted to measure the biological activity of the new compounds on the cerebral infarction therapy. The results in Figure 4 indicate that the content of coagulation factors in the model group was much higher than the control group, which is obviously higher than the control group. Under the treatment of compound 1, the content of coagulation factors in the serum was obviously reduced. However, compound 2 exhibited only a little influence on the content of coagulation factors in the serum.

3.3. Compound Obviously Inhibited the Inflammatory Response in the Brain. In the above research, we have proved that the new compound has excellent inhibitory activity on the content of coagulation factors in the serum. As the inflammatory response in the brain was also an important factor for the acute cerebral infarction, so, the real-time RT-PCR was further conducted, and the inflammatory response in the brain was determined. The results in Figure 5 suggest that the inflammatory response in the model group was higher than the control group. There was a significant difference between these two groups, with \( P < 0.005 \). However, after the treatment of compound 1, the inflammatory response in the brain was obviously inhibited, which is stronger than compound 2.

4. Conclusion

On the whole, we have produced two mixed-ligand CPs in success under a variety of solvent systems. The as-produced two CPs are structurally tested with the diffraction of single-crystal X-ray, which were in-depth characterized employing TGA, PXRD, and EA. The results of the ELISA assay indicated that compound 1 was much stronger than compound 2 on reducing the content of coagulation factors in the serum. The results of the real-time RT-PCR suggested that the inflammatory response in the brain was also inhibited by compound 1, but not compound 2. In the end, we draw this conclusion; compound 1 could significantly promote the activity of the Alteplase on the acute cerebral infarction therapy.

Data Availability

The IR spectrum for complexes 1-2 (Figure S1), the H-bond interactions in complex 1 calculated via PLATON (Figure S2), the H-bond interactions in complex 2 calculated via PLATON (Figure S3), and the information could be found in the supporting information file.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Figure S1: The IR spectrum for complexes. Figure S2: The H-bond interactions in complex 1 calculated via PLATON. Figure S3: The H-bond interactions in complex 2 calculated via PLATON. (Supplementary Materials)

References


