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Simple Coumarin-Based Fluorescent Probe for Recognition of Pd(II) and Its Live Cell Imaging

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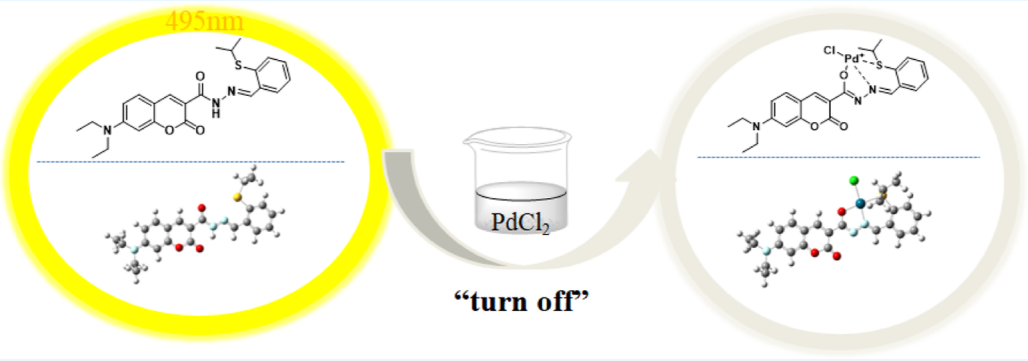

ABSTRACT: A simple coumarin hydrazine Schiff base bearing a thioether recognition fragment (compound **CBBS**) has been rationally designed and easily prepared. **CBBS** exhibited an excellent selectivity for Pd(II) and a low detection limit of 65 nM (S/N = 3). The fluorescence emission intensities of **CBBS** at 495 nm were linear to Pd(II) concentrations in a wide range from 0 to 80 μM. Moreover, **CBBS** has been well used in fluorescence imaging of Pd(II) in living A549 cells. **CBBS** as a simple coordination-based fluorescent probe will inspire the researchers to develop a polymer for selective detection and adsorption of Pd(II).

1. INTRODUCTION

Palladium(II), one of the biological toxicity metal ions, can cause eye irritations, skin cancer, and several cellular processes and biological activity disorders.[1] However, they have wide industrial applications, such as pharmaceuticals,[2] fuel cells,[3] manufacturing electrical products,[4] and so on. In this case, people are easily exposed to palladium(II) ions and susceptible to poisoning.[5] Moreover, owing to the maximum intake of palladium(II) ions being less than 15 μg/day/person, various research studies have emphasized that we should reduce the risk of contact palladium(II) ions.[6,7] Consequently, developing easily operational and efficient detection methods for palladium(II) ions in environmental and biological samples is rather necessary.

Recently, fluorometry has been one of the focuses in the detection of toxic matters.[8] Fluorescence-based methods for detecting palladium(II) ions are competitive with conventional techniques because we do not suffer from expensive instrumentation, complicated operations, and highly skilled operation skills.[9−11] Up to now, researchers have developed various fluorescent probes for recognition of palladium(II) ions.[12,13] However, most of them were reactive fluorescence probes.[14] As another type of fluorescence probe, coordination-based fluorescent probes are of great interest because they are privileged building blocks for preparing fluorescent polymers capable of selective adsorption of metal ions.[15] Additionally, coordination-based fluorescent probes containing a Schiff base fragment possess remarkable advantages, such as simple synthesis route, lower cost, and available raw materials.[16] Based on the above research background, we plan to develop a simple Schiff base for sensing palladium(II) ions.

Sulfide fragments can easily form a coordination bond between the sulfur atom and transition-metal ion, which is an attractive feature. For example, Faiges *et al.* disclosed that the thioether group of Ir/P,S-catalyst played a key role in directing the olefin coordination.[17] Diéguez *et al.* have highlighted the catalytic performance of phosphorus−thioether ligand libraries.[18] Mirica *et al.* reported one kind of heteroleptic mononuclear Pd(I) complex with high activity for C sp^3−H bond activation.[19] In that case, sulfur-containing recognition fragments would be beneficial for designing simple coordination-based fluorescent probes. However, the recognition abilities of simple fluorescent probes based on coumarin

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hydrazine Schiff bases containing thioether fragments for harmful heavy ions have not been systemically studied.

The construction of coordination-based coumarin fluorescent probes for detecting metal ions is a highly topical research field because of their ease of modification, relatively high-fluorescence quantum yield, and molar absorption coefficient.[20,21] However, there are few examples of coordination-based coumarin fluorescent probes for detecting palladium(II) ions.[22,23] Under this background, we begin to evaluate the sensing performance of an easily prepared coumarin Schiff base containing a thioether recognition fragment (compound **CBBS**) for palladium(II) ions. Furthermore, revealing the spectral properties of a coumarin-based fluorescence molecule with a thioether fragment could guide the design of the other coordination-based fluorescence probes for sensing metal ions.

2. RESULTS AND DISCUSSION

2.1. Spectroscopic Properties of CBBS. As illustrated in Figure 1, the UV−vis absorption spectrum of **CBBS** before and after the addition of 80 μM Pd(II) was investigated, respectively. Upon the addition of 80 μM Pd(II) (blue line), there was a marked decline in the UV absorption intensity (λ_{max} = 450 nm) of **CBBS**. Meanwhile, a redshift maximum absorption could be observed upon the addition of 80 μM Pd(II) to **CBBS**.

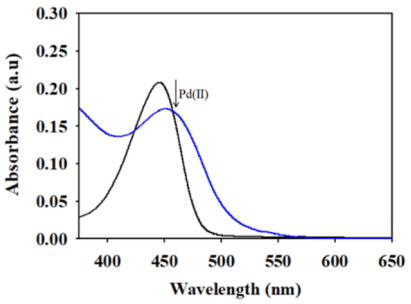

Figure 1. UV absorption changes of **CBBS** (10 μM) upon the addition of Pd(II) (80 μM) in phosphate-buffered saline (PBS)/dimethyl sulfoxide (DMSO) buffer solution (80/20, v/v, pH 7.4, 25 mM).

Next, we turned our attention to understand the fluorescence properties of **CBBS** with different concentrations of Pd(II) (0−80 μM) (Figure 2a). Obviously, the fluorescence emission intensity of **CBBS** at 495 nm gradually decreased with the increase of Pd(II) concentration. The fluorescence emission intensity of **CBBS** was at the lowest level, when 80 μM Pd(II) ions were added into the aqueous media containing **CBBS** (Figure 2a). The fluorescence emission intensity at 495 nm was proportional to the Pd(II) concentrations linearly in 0−80 μM (Figure 2b). The regression equations are F_1 = 7763.9 − 155.33 × [Pd^{2+}] (0−40 μM, R^2 = 0.990) and F_2 = 3118.4 − 30.66 × [Pd^{2+}] (40−80 μM, R^2 = 0.995). Moreover, the detection limit of **CBBS** was calculated to be 6.5 × 10^{-8} M (S/N = 3). These results suggested that **CBBS** as a fluorescent probe for detecting Pd(II) was acceptable.

2.2. Fluorescence Properties of CBBS toward Metal Ions. Furthermore, the fluorescent responses of **CBBS** toward different metal ions were investigated in PBS/DMSO buffer solution (80/20, v/v, pH 7.4, 25 mM). After adding 80 μM of the other ions (Mg^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Hg^{2+}, La^{3+}, Al^{3+}, Rh^{3+}, Cr^{3+}, Fe^{3+}, and Cd^{2+}) to the **CBBS** solution (10 μM), we did not observe the apparent changes of fluorescence intensity ratio (I/I_0) at 495 nm (Figure 3). This phenomenon indicated that **CBBS** had a high selectivity for detecting Pd(II).

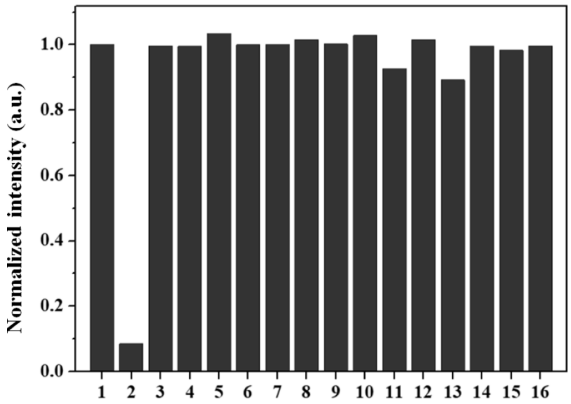

Figure 3. Fluorescence response of **CBBS** (10 μM) against various metal ions (80 μM); 1 = blank, 2 = Pd^{2+}, 3 = Mg^{2+}, 4 = Co^{2+}, 5 = Cu^{2+},6 = Mn^{2+},7 = Zn^{2+}, 8 = Ni^{2+}, 9 = Pb^{2+}, 10 = Hg^{2+}, 11 = La^{3+}, 12 = Al^{3+}, 13 = Rh^{3+}, 14 = Cr^{3+}, 15 = Fe^{3+}, and 16 = Cd^{2+}; λ_{em} = 495 nm.

2.3. Effect of pH and Response Time of CBBS. As shown in Figure 4, in a pH range of 4.0−10.0, the fluorescence intensity of **CBBS** was high (solid line). Obviously, once 80 μM Pd(II) was added to the PBS/DMSO buffer solution containing **CBBS**, the fluorescence intensity of **CBBS** solution

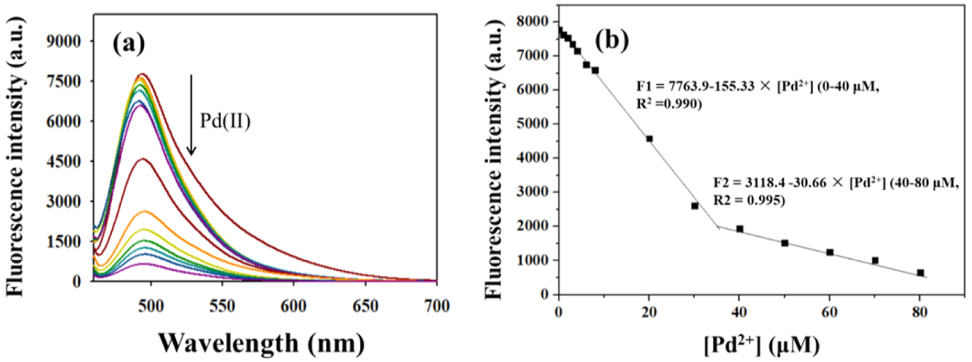

Figure 2. (a) Fluorescence spectra of **CBBS** (10 μM) upon the addition of Pd(II) (0−80 μM) in PBS/DMSO buffer solution (80/20, v/v, pH 7.4, 25 mM). (b) Fluorescence emission intensity of **CBBS** at 495 nm *vs* the concentration of Pd(II) (λ_{ex} = 450 nm).

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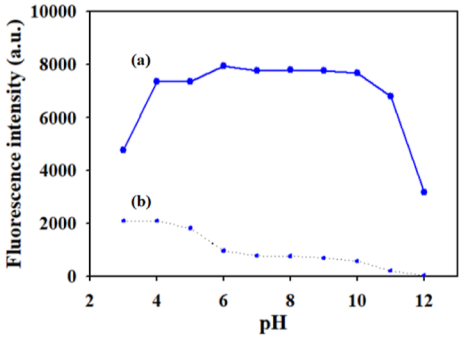

Figure 4. Effect of pH values on the fluorescence intensity of **CBBS** (10 μM) in the absence (solid line) and presence of 80 μM Pd(II) (dashed line).

had a largest decrease in the range of pH 4−10 (dashed line). This finding showed that **CBBS** as a fluorescence probe for Pd(II) could keep a stable detection performance within a wide range of pH values.

For preliminary understanding the kinetic property of **CBBS** after adding Pd(II), the response time was evaluated (Figure 5). We first confirmed that the fluorescence intensity of **CBBS**

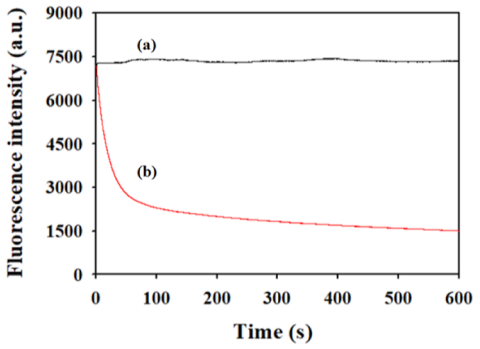

Figure 5. Time profile of **CBBS** (10 μM) in the absence (line a, black) and presence of 80 μM Pd(II) (line b, red) in PBS/DMSO buffer solution (80/20, v/v, pH 7.4, 25 mM).

itself was stable (line a). Also then, it was observed that when 80 μM Pd(II) was added to the detection system, the fluorescence intensity of **CBBS** at 495 nm decreased rapidly (line b) and tended to be stable in a short time (600 s). This findings suggested that **CBBS** could detect Pd(II) quickly.

2.4. Bing Mechanism and DFT Calculation. As shown in Figure 6, the electrospray ionization-mass spectrometry (ESI-MS) spectra of complex **CBBS**−Pd(II) showed a peak at m/z 578.0491, which matched the calculated value of m/z 578.0486 ($C_{24}H_{27}ClN_3O_3SPd^+$). For gaining the other evidence about the binding model, Job's plot of **CBBS**−Pd(II) was also performed (Figure S3). An intermediate fluorescence intensity at 495 nm was observed when the molecular fraction of Pd(II) was 0.5, indicating the 1:1 binding of **CBBS** with Pd(II).

Beyond that, we have used the long-range separated hybrid functional CAM-B3LYP with def2-SVP basis to optimize the geometry and calculate the excited state property of **CBBS** and complex **CBBS**−Pd(II), respectively (Figure 7). The calcu-

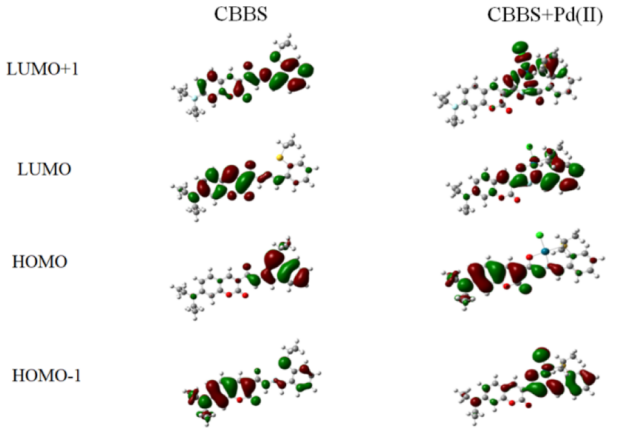

Figure 7. Frontier molecular orbital profiles of **CBBS** and complex **CBBS**−Pd(II) obtained from the density functional theory (DFT) (CAM-d3LYP/def2SVP) calculations.

lated oscillator strength (f) of **CBBS** was 1.27, which was much larger than the 0.01 of **CBBS**−Pd(II). This data indicated that Pd(II) could quench fluorescence of **CBBS** from the perspective of theoretical calculation. According to the above data, a possible binding model of **CBBS** with Pd(II) was proposed (Scheme 1).

2.5. Imaging in Cells. For checking the potential bioapplications of **CBBS**, imaging of Pd(II) ions in A549 cells was preliminarily studied (for the experiment details, see the Supporting Information). In the yellow channel (470−600 nm), a fluorescence signal was observed when living A549 cells were incubated with 10 μM **CBBS** for 30 min (Figure 8a). When 80 μM Pd(II) ions were added to A549 cells pretreated with N-ethylmaleimide (NEM) and preincubated with **CBBS** successively, a weak fluorescence signal was observed an hour later (Figure 8c). In addition, the toxicity of **CBBS** on A549 cells was investigated using the 3-[4,5-dimethylthiazol-2-yl]-2,5

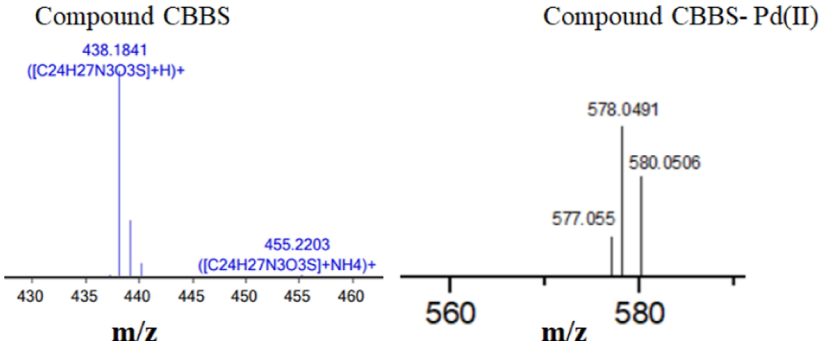

Figure 6. ESI-MS spectra of **CBBS** and complex **CBBS**−Pd(II).

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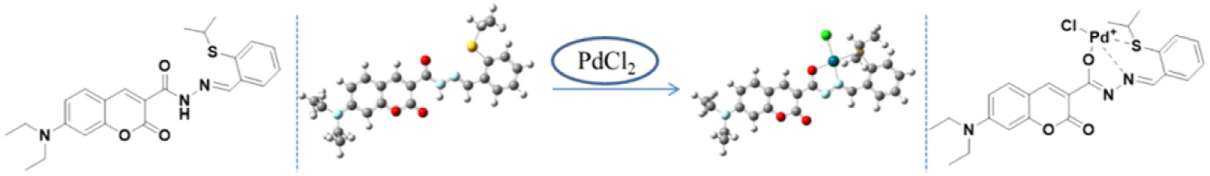
Scheme 1. Proposed Binding Mechanism of CBBS toward Pd(II)



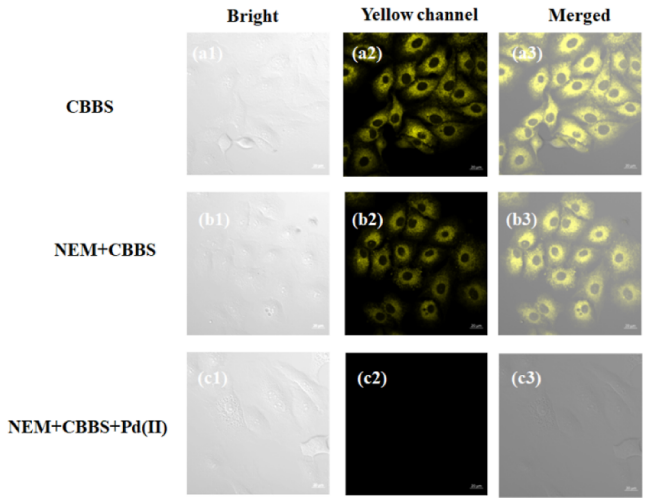


Figure 8. Fluorescence images of A549 cells incubated with **CBBS** for 30 min. (a1−a3) Fluorescence images of A549 cells pretreated with NEM (1.0 mmol/L) for 30 min and then incubated with **CBBS** for 30 min (b1−b3) and those first incubated with NEM and **CBBS**, respectively, for 30 min, and then incubated with Pd(II) for 60 min (c1−c3).

diphenyl tetrazolium bromide method (Figure S5). After 12 or 24 h of incubation with 10 μM **CBBS**, the survival rate of A549 cells is still very high. These results indicated that **CBBS** as a coumarin-based fluorescent probe for imaging of Pd(II) ions in A549 cells was practicable.

3. CONCLUSIONS

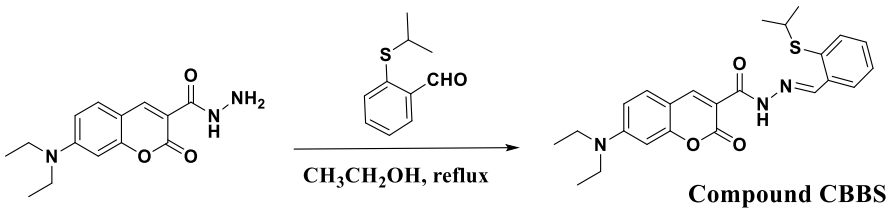
A simple coumarin Schiff base-based fluorescent probe named **CBBS** was successfully developed. The linear fashion of the fluorescence intensity decline of **CBBS** with the concentration of Pd(II) could be applied in the quantification of Pd(II) in aqueous solution. A possible binding model of **CBBS** with Pd(II) was studied *via* high-resolution (HR)-MS, Job's plots, and DFT calculation. Moreover, **CBBS** could be applied in identifying Pd(II) in living A549 cells. This study has enriched the luminous family of coumarin-based Schiff bases bearing a thioether fragment.

4. EXPERIMENTAL SECTION

4.1. Reagents and Apparatus. 2-Fluorobenzaldehyde, isopropyl mercaptan, 4-(diethylamino)salicylaldehyde, and ethanol were provided by Energy-Chemical Reagent Co., Ltd. A 5.0 mM stock solution of $PdCl_2$ and $RhCl_3{\cdot}3H_2O$ was both prepared in the cosolvent solution (brine/methanol, 1:3, v/v). The solutions of various metal ions were obtained from $MgCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $MnSO_4$, $Zn(NO_3)_2{\cdot}7H_2O$, $NiSO_4{\cdot}6H_2O$, $PbCl_2$, $HgCl_2$, $LaCl_3{\cdot}7H_2O$, $Al_2(SO_4)_3$, $CrCl_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, and $CdCl_2{\cdot}2.5H_2O$ using ultrapure water. The melting point of **CBBS** was tested in an X-4B instrument (Yidian Physical Optical Instrument Co., Ltd, China). NMR measurements were performed on a Bruker AVB-400 MHz spectrometer (Switzerland). The HR-MS analyses were carried out on a Bruker solanX 70 Fourier transform (FT)-MS. UV−vis absorption spectra were investigated by a Shimadzu UV-2450 (Japan). Fluorescence properties of **CBBS** were studied by a Hitachi F-7000 (Japan) with setting both excitation slit and emission slit to 5 nm. Frontier molecular orbital profiles of **CBBS** and **CBBS**−Pd(II) complexes based on the DFT (CAM-d3LYP/def2SVP) calculations were conducted in the framework of the Gaussian 09 program.

4.2. Preparation of Compound CBBS. The synthesis of compound **CBBS** is summarized in Scheme 2. 2-(Isopropylthio)benzaldehyde and 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbohydrazide (coumarin hydrazine) were easily synthesized according to previously reported methods, respectively.[24,25] Compound **CBBS** was synthesized by a condensation reaction of coumarin hydrazine (1.4 g, 5.0 mmol) with 2-(isopropylthio)benzaldehyde (1.0 g, 5.5 mmol) in 20.0 mL of absolute ethanol. Under nitrogen, the reaction solution refluxed in an oil bath for 12 h and cooled to room temperature. Finally, a yellow solid compound **CBBS** was obtained through column chromatography (dichloromethane as the eluent) (1.9 g, 86% yield). m.p. 187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 11.92 (s, 1H), 8.78 (s, 1H), 8.23 (s, 1H), 7.51−7.49 (d, J = 8 Hz, 2H), 7.34−7.33 (m, 2H), 6.74−6.72 (m, 1H), 6.59 (s, 1H), 3.51−3.46 (m, 4H), 3.30−3.24 (m, 1H), 1.35−1.26 (q, 6H), 1.25−1.20 (q, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 162.8, 159.9, 157.8, 152.9, 149.2, 147.2, 136.0, 135.4, 131.4, 130.3, 127.9, 127.6, 127.5, 110.3,

Scheme 2. Syntheses of Compound CBBS



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109.1, 108.7, 96.7, 45.2, 40.1, 23.1, 12.4; HR-MS (ESI$^+$, m/z): $[M + H]^+$ calcd for $C_{24}H_{28}N3O_3S$, 438.1846; found, 438.1841.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04626.

^{1}H NMR, ^{13}C NMR, and HR-MS spectra for **CBBS**; Job's plot diagram of **CBBS** for Pd^{2+}; experimental process of A549 cell culture; survival of A549 cells in 10 μM **CBBS** in 12 or 24 h; and dominant conformation of **CBBS** and **CBBS**−Pd(II) (PDF)

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Notes

The authors declare no competing financial interest.

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