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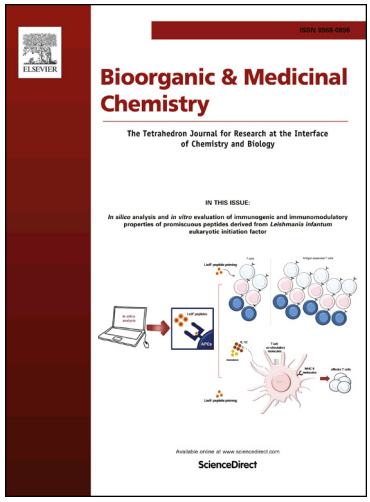

A water-soluble near-infrared fluorescent probe for specific Pd^{2+} detection

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Graphical Abstract

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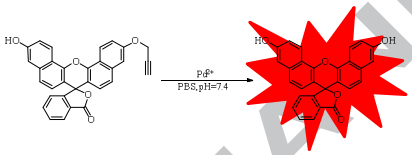

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A water-soluble near-infrared fluorescent probe for specific Pd^{2+} detection

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ABSTRACT

Palladium (Pd) is widely used in chemistry, biology, environmental science *etc.*, and Pd^{2+} is the most plenitudinous oxidation state of the Pd that can exist under physiological conditions or in living cells, which could have adverse effects on both our health and environment. Thus, it is of great significance to monitor the changes of Pd^{2+}. Hence, a novel near-infrared fluorescent probe **M-PD** has been developed for selective detection of Pd^{2+} based on naphthofluorescein in this work. The result demonstrated that **M-PD** exhibited favorable properties for sensing Pd^{2+} such as excellent water solubility, high selectivity and sensitivity. And the limit of detection was estimated as 10.8 nM, much lower than the threshold in drugs (5-10 ppm) specified by European Directorate for the Quality Control of Medicines. More importantly, detection and recovery experiments of Pd^{2+} in aspirin aqoeous solution and soil are satisfactory. In addition, **M-PD** has also been successfully used for near-infrared fluorescence imaging of Pd^{2+} in living cells, indicating that the probe has better feasibility and application potential in the determination of Pd^{2+}.

1. Introduction

With the development of modern industry, heavy and transition-metal ions have received intense attention in many fields such as chemistry, biology, and environmental science.[1,2] Among them, palladium (Pd), a rare inner transition metal, is enormously used as efficient catalysts in the fields of material science, drug synthesis, organic catalytic reactions, automobile exhaust disposing and fuel cells, *etc.*[2] In addition, Pd is also widely used to prepare jewellery, electric equipment and dental materials.[3] However, even after purification of the final products, the residual Pd contaminates are still typically at a level of 300-2000 ppm, which is drastically higher than the specified threshold in drugs (5-10 ppm).[4] It is worthy note that, Pd species can disturb many biological processes and lead to hard-to-ignore health hazard through tightly binding to biomolecules such as thiol-containing amino acids, protein, DNA, RNA and vitamins *etc.*[5-7] In particular, Pd^{2+} is the most plenitudinous oxidation state of the Pd that can exist under physiological conditions or in living cells. Hence, development of efficienct methods for detecting and imaging Pd^{2+} is important for environment safe and human health.

Up to now, there are many traditional methods reported for the detection of Pd^{2+},[8,9] such as atomic absorption/emission spectrophotometry, solid phase microextraction-high performance liquid chromatography, ion-coupled plasma emission-mass spectrometry, X-ray fluorescence and *etc.*[10-12] Unfortunately, they usually suffer from the expensive instruments, time-consuming sample-pretreatment and require well-trained individuals, which restricted their further practical applications.[13,14]

In stark contrast, optical detection methods, particularly fluorescence probes, show unique potential for the development of highly sensitive and selective as well as relatively simple analysis protocols. In recent years, a diverse kinds of fluorescent probes for Pd^{2+} detection have been developed owing to their high sensitivity and easy operation.[15-25] To date, there are four main reaction mechanism to design and develop these probes, including typical-metal binding, Pd-catalyzed fluorophore formation,[26] Pd-catalysed Tsuji-Trost and Pd-catalysed depropargylation. The probe based on metal binding mechanism was usually difficult to achieve with high selectivity and sensitivity for Pd^{2+}.[27-29] And Pd fluorescent sensor based on Pd-

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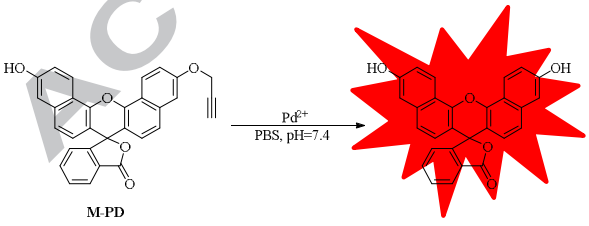
catalyzed Tsuji-Trost reaction needed to add additional reducing agent such as PPh_3, TFP (tri-2-furylphosphine) or TFP–$NaBH_4$ for conversion of Pd^{2+} to Pd^0 in detection of Pd^{2+}.[30] To avoid these problems, the Pd-catalysed depropargylation mechanism was more favored for the design of Pd^{2+} fluorescent probes.[31-33] However, most of these probes work in a mixture of water and organic solvent, which will somehow restrict their application in the environmental and biological field. Thus, development of probes with excellent water solubility is of great importance.[34-36] For example, in 2016, Wang *et al.* reported a fluorescent and colorimetric probe containing oxime-ether for Pd^{2+} in pure water and living cells;[37] Most recently, Hou's group reported a coumarin-based colorimetric and fluorescent dual probe for Pd^{2+} that can be used in live cells.[38] While, these probes need the ultraviolet or visible light to excite, which severely limits in biological applications because the fluorescence imaging in the visible region would be easily disturbed by auto-fluorescence of living systems.[39-41] In comparison, near infrared (NIR) fluorescent probes, which own deeper tissue penetration and thus can reduce auto-fluorescence efficiently. Therefore, a potential approach is to develop NIR fluorescent probes for the detection of Pd^{2+}.[42] Recently, Wang's group has designed a NIR fluorescent probe for Pd, which exhibits high sensitivity and selectivity toward both Pd^0 and Pd^{2+}. Unfortunately, this probe has a main drawback of poor water solubility, which is a disadvantage for bio-imaging application.[43] Zhang's group reported a fluorescent probe for Pd. Although this probe emits in the red light region, it cannot be used for monitoring Pd in living cells owing to the need of high proportion of organic solvents.[44]

To solve these, we designed and synthesized a new fluorescent Pd^{2+} probe **M-PD** with emission in the NIR region. The probe is composed of naphthofluorescein as the fluorophore and the terminal propargyl moiety which has been demonstrated to be favorable for selective palladium recognization45 as the recognition unit (Scheme 1). In the absence of any analyte, the probe **M-PD** has almost no fluorescence due to the protection of hydroxyl group of naphthofluorescein by propargyl group. Upon the addition of Pd^{2+}, the depropargylation reaction occurs and thus the protected hydroxyl group of **M-PD** is liberated,45 which lead to the significant enhancement of fluorescence. In detection of Pd^{2+}, **M-PD** displays high selectivity, sensitivity and excellent water solubility. Most importantly, detection and recovery experiments of Pd^{2+} in aspirin aqoeous solution and soil are satisfactory. In addition, **M-PD** could also be used to monitor Pd^{2+} in living cells (human hepatocellular carcinoma cell HepG-2 was chosen as an example). All of these performances make **M-PD** appropriate for potential application in environment or living systems.



Scheme 1. Recognition mechanism of **M-PD** toward Pd^{2+}.

2. Experimental sections

2.1. Reagents and apparatus

All reagents were purchased from commercial suppliers and used without further purification. All the organic solvents were analytical grade. Deionized water was used for all the measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 AVANCE III spectrometer with chemical shifts reported in ppm at room temperature (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, Germany). High resolution mass spectrum (HRMS) was obtained with Thermo Fisher LCQ Fleet mass spectrometer (USA).

All spectrographic measurements were performed in 10 mM phosphate buffered saline (PBS) (pH 7.4). The pH of the testing systems was determined by a PHS-3C pH Meter (China). Absorption spectra were measured with a Shimadzu UV-1750 UV-vis spectrometer (Japan). Luminescence spectra were collected by using a Shimadzu RF-5301 fluorescence spectrometer (Japan). Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37°C. Cell toxicity tests were tested by microplate reader (KHB ST-360). The confocal laser microscope data were acquired using a confocal fluorescence microscope (Nikon A1R). Aspirin was purchased from Northwest A&F University hospital and dissolved in PBS for further use. Soil was obtained in Northwest A&F University. And the soil was pretreated with acid before detection according to the literatures.[56-58]

All of the experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Northwest A&F University.

*2.2. Synthetic procedures of probe (**M-PD**)*

Synthesis of Compound **M**: According to the literature,[46] phthalic anhydride (0.890 g, 6.0 mmol) and 1,6-dihydroxynaphthalene (2.40 g， 15 mmol) were added in a round-bottom flask. After adding methanesulfonic acid (15 mL), the mixture was refluxed for 12 h at 135□. Next, the mixture was poured into ice-water and filtered. The crude product was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH = 125:1) to give compound **M** as a aubergine solid. Yield: 0.983 g (37.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 2H), 8.70 (d, J = 9.1 Hz, 2H), 8.09 (m, 1H), 7.76 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.37 (dd, J = 9.1, 2.4 Hz, 2H), 7.30 (d, J = 6.7 Hz, 1H), 7.21 (d, J = 2.4 Hz, 2H), 6.71 (d, J = 8.8 Hz, 2H).

Synthesis of Compound **M-PD**: According to the literature,[47] **M** (0.432 g，1.0 mmol) and potassium carbonate (0.207 g，1.5 mmol) were added in a round-bottom flask. After adding anhydrous acetonitrile (15 mL), the mixture was stirred for 1 h at 50□. Next, 3-bromopropyne (83 μL，1.1 mmol) was added and the mixture was refluxed for 6 h and then extracted with dichloromethane. The crude product was purified by silica gel column chromatography (CH_2Cl_2/ $CH_3COOC_2H_5$ = 150:1) to give compound **M-PD** as a light yellow solid. Yield: 0.263 g (55.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.77 (d, J = 9.1 Hz, 1H), 8.71 (d, J = 9.1 Hz, 1H), 8.10 (dd, J = 6.2, 1.9 Hz, 1H), 7.81 – 7.73 (m, 3H), 7.59 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.49 – 7.45 (m, 3H), 7.37 (dd, J = 9.0, 2.4 Hz, 1H), 7.31 (dd, J = 6.2, 1.5 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 6.72 (d, J = 8.7 Hz, 1H), 4.99 (d, J = 2.3 Hz, 2H), 3.64 (t, J = 2.3 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.83, 157.40, 156.86, 153.22, 146.02, 145.93, 135.97, 135.83, 135.41, 130.33, 125.71, 124.80, 124.49, 124.20, 123.90, 123.80, 123.21, 122.75, 119.35, 119.30, 118.50, 117.27, 110.70, 109.44, 109.37, 108.32, 82.99, 78.88, 78.60, 55.73. HRMS: $[M+H]^+$, calculated for $[C_{31}H_{18}O_5]$: 471.1227; found: 471.12286.

2.3. Analytical procedure

All experiments were performed in an absolute PBS solution (pH 7.4, 10 mM). In the selectivity experiments, the test samples were prepared by adding an appropriate amount of the metal ions stock solution (50 μM Pd^{2+} and 100 μM other metal ions) to a 3 mL solution of **M-PD** (10 μM). In the titration experiment, a solution of **M-PD** (10 μM) was prepared at room temperature with the addition of appropriate amount of $PdCl_2$ solution, which was added to the quartz cuvette using a micropipette. Spectroscopic data were recorded upon the addition of analytes at room temperature. Spectroscopic data were recorded 30 min after the addition of the metal ions. And the fluorescence emission spectra of the result solutions were recorded at an excitation wavelength of 610 nm (unless otherwise noted, all spectral were measured according to this method).

The detection limit was calculated based on the fluorescence titration according to the literature.[48] In the absence and presence of Pd^{2+}, the fluorescence emission spectra of **M-PD** were measured three times for each point and the standard deviation of blank measurement was achieved. To gain the slop, the fluorescence intensity at 668 nm was plotted to the concentration of Pd^{2+}. So the detection limit was calculated with the following equation:

Detection limit = 3σ/k

Wherein σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity versus Pd^{2+} concentration.

In real sample analysis, according to the literatures,[62-64] 10 g of surface soil was added to polytetrafluoroethylene (PTFE) beakers containing 50 mL aqua regia. The mixture was heated to near dryness, re-dissolved into ultrapure water, filtrated to remove suspended matters, sealed and stored in 50 mL PTFE bottles for use. Aspirin aqoeous solution was prepared by dissolving different amount of aspirin in ultrapure water. Then, different amount of Pd^{2+} was added into soil or aspirin samples for further use. A solution of **M-PD** (10 μM) was prepared at room temperature with the addition of appropriate amount of pretreated Pd samples, which was added to the quartz cuvette using a micropipette. Spectroscopic data were recorded upon the addition of analytes at room temperature.

2.4. Cell cultures, cytotoxicity assay and fluorescence imaging

HepG-2 cells were cultured in 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete 1640) in 5% CO_2 at 37 °C. For cell viability study, HepG-2 cells were seeded in 96-well microplates at a density of 1×10^5 cells/mL in 100 μL complete 1640 medium. After 24 h of cell attachment, plates were washed with 100 μL/well PBS and then cells were cultured in medium with various concentrations (2.5-20 μM) of **M-PD** for 24 h. 10 μL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37°C for 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple products were lysed in 100 μL dimethyl sulfoxide (DMSO). The plate was shaken for 10 min and the absorbance was measured at 490 nm using a microplate reader (KHB ST-360).

For fluorescence imaging, HepG-2 cells were seeded in 35-mm dishes and allowed to adhere for 24 h. Then the cells were treated with 10 μM **M-PD** and incubated at 37°C for 1 h. After removing the culture medium and washing twice with PBS, fluorescence images of cells were captured. Complete 1640 were added to the cell culture, which was then treated with a 20 μM Pd^{2+} and incubated at 37°C for an additional 30 min. After washing twice with PBS, fluorescence images of the cells were captured.

3. Results and discussion

*3.1. Synthesis of probe **M-PD***

The detailed synthetic routine of probe **M-PD** is outlined in Scheme S1 in the Supporting Information. By two simple steps, phthalic anhydride and 1,6-dihydroxynaphthalene in the solution of methanesulfonic acid were refluxed for 12 h giving compound **M**. Then, compound **M-PD** was obtained by the reaction of compound **M** with potassium carbonate and 3-bromopropyne in anhydrous acetonitrile, which was confirmed by ^1H NMR, ^{13}C NMR and HRMS (Fig. S1-S4).

*3.2. Spectral properties of **M-PD***

The spectroscopic properties of **M-PD** were assessed in an absolute PBS at pH 7.4. **M-PD** (10 μM) displayed one major absorption band centered at 578 nm. This band was increased and had a silght red-shifted after the addition of 7 equiv of Pd^{2+} (Fig. S7). In the absence of Pd^{2+}, with 610 nm excitation, the **M-PD** in PBS solution emitted weak fluorescence, which was mainly because the propargyl group in **M-PD** trammeled the hydroxyl group of naphthofluorescein. While with the addition of Pd^{2+} ions (0-7 equiv), the fluorescence intensity at 668 nm gradually enhanced, attributing to the disappearance of propargyl group and the liberation of protected hydroxyl group (Fig. 1a).

Moreover, Fig. 1b showed that the fluorescence intensity increased linearly with the increasing Pd^{2+} concentration from 0.0 to 70 μM (R^2 = 0.998), and the detection limit was measured to be 10.8 nM. Compared with previous probes for Pd^{2+} detection based on Pd-catalysed depropargylation mechanism (Table 1),[27,35,49-61] **M-PD** not only can work well in an absolute PBS solution (Fig. S8) but also exhibit low detection limit and NIR emission, indicating that **M-PD** could be used as an effective tool for the sensing of Pd^{2+} in environmental and biological fields.

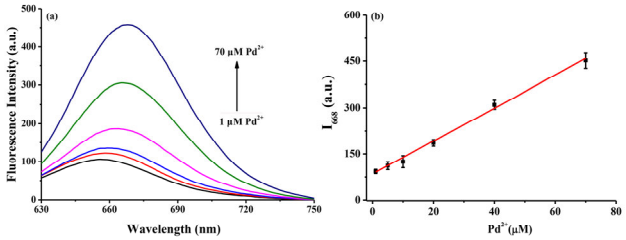


Fig. 1. Fluorescence titration studies of **M-PD**. (a) Fluorescence spectra of **M-PD** (10 μM) upon addition of Pd^{2+} (1-70 μM) in PBS buffer; (b) The linear relationship between the fluorescence intensity and the concentration of Pd^{2+}. All data were collected at 30 min after the addition of Pd^{2+} (n=3).

3.3. Selectivity for Pd^{2+} detection

For a new fluorescent probe, it is very important to own highly selective response to the target species over other potentially competing species, which is necessary for potential application in complex biological and environmental samples. Therefore, the selectivity studies of **M-PD** toward various metal ions were investigated and the results are shown in Fig. 2. There was no notably fluorescence change in the presence of the common cations, such as Ag^+, Al^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}. However, a much larger fluorescence enhancement was observed for **M-PD** upon addition of Pd^{2+}. These results clearly demonstrate that **M-PD** is a highly selective fluorescent probe for Pd^{2+}, and also verify that the depropargylation is specific toward Pd^{2+}.[45]

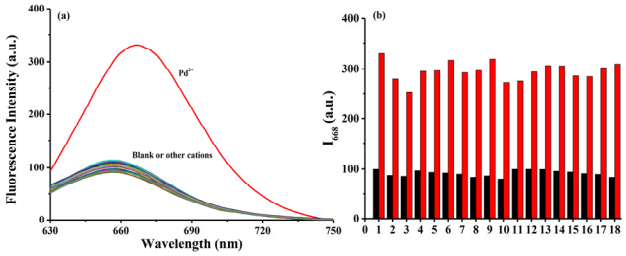
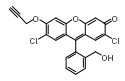
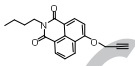
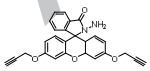
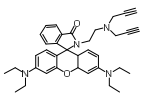
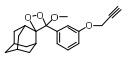
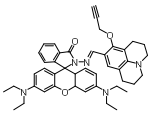
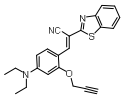
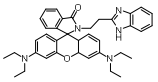
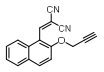
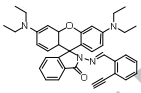
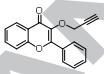
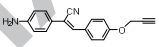
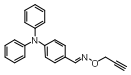
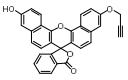


Fig. 2. Competitive experiments for selectivity. (a) Fluorescence responses of **M-PD** (10 μM) to Pd^{2+} (50 μM) and other cations (100 μM for each) in PBS (10 mM, pH 7.4). (b)Fluorescence intensity changes of **M-PD** (10 μM) upon the addition of various metal ions (100 μM): 1. Blank, 2. Ag^{+}, 3. Al^{3+}, 4. Ba^{2+}, 5. Ca^{2+}, 6. Cd^{2+}, 7. Co^{2+}, 8. Cu^{2+}, 9. Fe^{2+}, 10. Fe^{3+}, 11. Hg^{2+}, 12. K^{+}, 13. Li^{+}, 14. Mg^{2+}, 15. Mn^{2+}, 16. Ni^{2+}, 17. Pb^{2+}, 18. Zn^{2+}, in the presence of Pd^{2+} (50 μM) in PBS. The black bars represent the fluorescence response of **M-PD** and competing ions. The red bars represent the subsequent addition of 50 μM Pd^{2+} to the above solutions. All data were measured after 30 min.

3.4. Proposed mechanism

The recognition mechanism of **M-PD** to Pd^{2+} could be reasonably explained by the catalytic mechanism of Pd^{2+} with the propargyl group (Scheme 1). The ^{1}HNMR spectroscopic analysis provided the evidence for the binding pattern between **M-PD** and Pd^{2+}. The propargyl group of **M-PD** trammeled the hydroxyl group of **M-PD**, hence the probe had weak fluorescence. After the addition of Pd^{2+}, the propargyl group of the **M-PD** in the upfield disappeared (Fig. S5). Furthermore, another hydroxyl group was formed in the downfield compared to the ^{1}HNMR spectroscopic of **M-PD** (Fig. S2). These results were ascribed to the formation of compound **M**, following with fluorescence changed from colorless to red. To further explore the mechanism, addition of Pd^{2+} to **M-PD** was subsequently analyzed by MS. As shown in Fig. S6, a peak at *m/z* 433.15 corresponding to **M** was observed, demonstrating the depropargylation reaction of **M-PD** catalyzed by Pd^{2+}occurred.

Table 1. Comparisons of the proposed probe with previous reports.

Structure of probe	Solvent	Detection Limit	Emission Wavelength	Ref.
	water containing 10% CH_3CN	30 nM	520 nm	21
	PBS	70 nM	553 nm	43
	CH_3CN : H_2O (1 : 4, v/v)	87 nM	517 nm	44
	PBS containing 10% (v:v) CH_3CN	-	492 nm	45
	H_2O : CH_3CN (1 : 1, v/v)	40 nM	472 nm	29
	CH_3CN : H_2O (1 : 1, v/v)	-	574 nm	46
	DMSO : H_2O (95 : 5, v/v)	88 μM	466 nm	47
	CH_3CN–H_2O	156 nM	570 nm	48
	EtOH : H_2O (1 : 1, v/v)	21 nM	569 nm	49
	PBS containing 50% EtOH	93 nM	542 nm	50
	PBS containing 1.0% EtOH	53 nM	457 nm	51
	EtOH : H_2O (1 : 1, v/v)	191 nM	530 nm	52
	pure water containing 1 mM Triton X-100	0.9 μM	526 nm	53
	EtOH : HB, (10 : 90, v/v)	9.3 ppM	517 nm	54
	EtOH : Hepes (1 : 2000, v/v)	0.47 μM	423 nm	55
	PBS	10.8 nm	668 nm	**This work**

3.5. Analysis of real samples and recovery test

To validate the feasibility of the method when applied to real samples, determination of Pd^{2+} in aspirin aqoeous solution and soil were conducted based on the aforementioned procedures. Fig. 3 showed that no Pd^{2+} was found in aspirin aqoeous solution or soil samples. Taking these two samples as substrates, standard addition and recovery tests were further conducted. Known amounts (2, 6, 10 μM, which were all lower than the specification in drugs stated by European Directorate for the Quality Control of Medicines[4]) of Pd^{2+} were spiked into samples prior to pretreatment (in a simplified pretreatment, we need only to extract soil samples with acid and filter it)[62-64] and recovery results were conducted. The results indicate that **M-PD** offer ideal sufficient sensitivity and accuracy, which is satisfactory for real sample analysis.

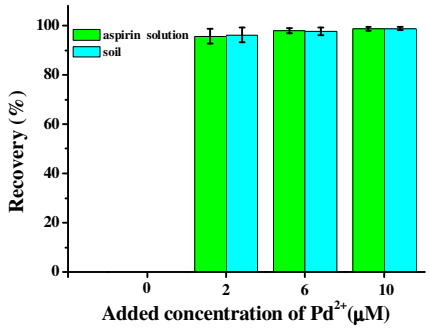


Fig. 3. Detection and recovery of Pd^{2+} in aspirin solution and soil (n = 3).

3.6. Cellular imaging

Before the application of **M-PD** in cell imaging, the cytotoxicity of **M-PD** was examined by standard MTT assay. As shown in Fig. S9, the cell viability was still over 78% after 24 h even when the concentration of **M-PD** reached up to 20 μM, which demonstrates the low cytotoxicity of the probe. Then in order to evaluate the cell permeability of **M-PD** and capability of **M-PD** for the detection of Pd^{2+}, living cells imaging was carried out (Fig. 4). HepG-2 cells were incubated with 10 μM of **M-PD** for 1 h, and then treated with 20 μM of Pd^{2+} for 30 min. The fluorescence images of HepG-2 cells were recorded before and after addition of Pd^{2+}. As shown in Fig. 4a, in the absence of Pd^{2+}, free **M-PD** showed weak fluorescence signals in living cells. Whereas, after incubation with Pd^{2+}, obvious enhanced red fluorescence was observed in living cells (Fig. 4b). These data demonstrate that **M-PD** has good cell-membrane permeability and could also be used to monitor the changes of Pd^{2+} level in living cells.

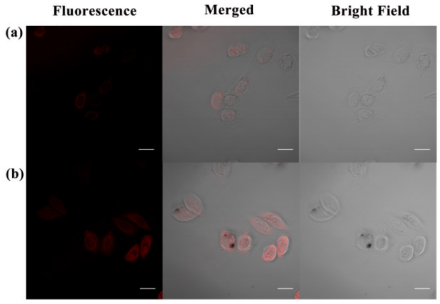


Fig. 4. CLSM images of HepG-2 cells incubated with **M-PD** (10 μM) for 1 h (a), followed by incubation with 20 μM Pd^{2+} for 30 min (b). Scale bar: 20 μm.

4. Conclusions

In summary, a naphthofluorescein-based NIR fluorescent probe **M-PD** was developed for selective recognition of Pd^{2+}. The results demonstrate that **M-PD** exhibits excellent water solubility and a high sensitivity for Pd^{2+} detection with the detection limit of 10.8 nM, which is much lower than the threshold in drugs (5-10 ppm) stated by European Directorate for the Quality Control of Medicines. **M-PD** has been successfully used for NIR fluorescence detection of Pd^{2+} in aspirin aqoeous solution and soil, imaging of Pd^{2+} in living cells, providing a promising strategy for the detection of Pd^{2+} in biological and environmental systems.

Bibliography

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