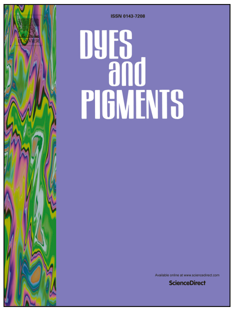

Highly sensitive and selective ESIPT-based fluorescent probes for detection of Pd^{2+} with large Stocks shifts

Tiantian Chen, Tingwen Wei, Zhijie Zhang, Yahui Chen, Jian Qiang, Fang Wang, Xiaoqiang Chen

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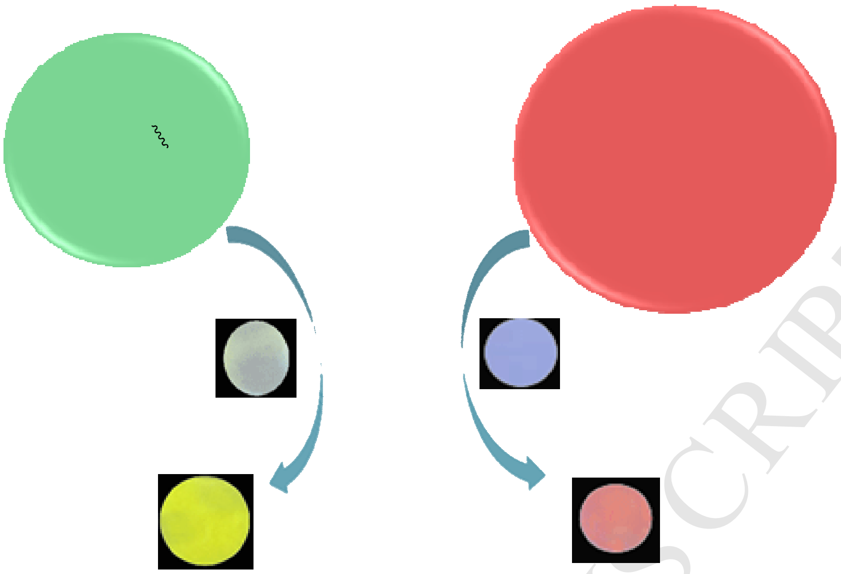

Two 2-(2’-hydroxyphenyl)benzothiazole-based fluorescent probes **1** and **2** were designed and exhibited strong fluorescence in short duration upon exposure to Pd^{2+}. The portable test strips were also prepared by direct deposition of the probe molecules onto the filter paper to detect the lowest possible ppm levels of Pd^{2+}.

Highly sensitive and selective ESIPT-based fluorescent probes for detection of Pd^{2+} with large Stocks shifts

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Abstract Highly sensitive and selective fluorescent probes **1** and **2** for detection of palladium ions (Pd^{2+}) were synthesized based on excited-state intramolecular proton transfer process (ESIPT) using 2-(2'-hydroxy-)benzothiazole moiety. Different metal ions were used to optimize their sensitivity and selectivity but best results were obtained with Pd^{2+}. Probe **1** produced green fluorescence with Stokes shift of about 163 nm upon addition of Pd^{2+}, whereas probe **2** displayed red fluorescence with a large Stokes shift of about 217 nm under similar conditions. The detection limits of **1** and **2** to Pd^{2+} were found to be 285 nM and 14.6 nM, respectively. Mechanistic aspects of probes **1** and **2** were discussed to account for the optical changes leading to the strong ESIPT fluorescence in very short duration. Furthermore, the portable test strips were also prepared by direct deposition of the probe molecules onto the filter paper to detect the lowest possible ppm levels of Pd^{2+}.

Keywords: Fluorescent probe; ESIPT; Palladium; Large Stocks shifts; Benzothiazole

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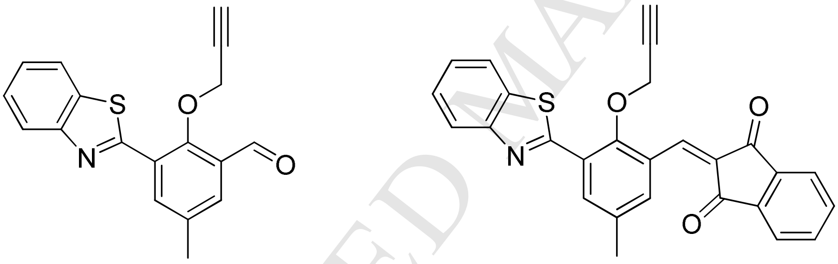
1. Introduction

Palladium (Pd) has been widely used as key materials in numerous scientific areas including catalytic converters, medical instruments, dental alloys, fuel cells, electronics and jewelry.[1-4] Pd-catalyzed reactions comprise an important area of research interests including transformations for synthesis of functional molecules and drugs.[5] However, their frequent use has resulted in a high level of residual palladium in water systems and soil.[6-8] Palladium may form complexes with thiol-containing amino acids, proteins, DNA, RNA, and vitamins to disrupt the physiological processes of human life cycle along with other serious health problems, and hence, poses a threat to human health. [9-11] The extensive use of palladium increases the risk associated with human health.[12, 13] Considering all these hazardous effects of palladium, European Agency for the Evaluation of Medicinal Products (EMEA) has set the threshold limit of palladium found in drugs as 5–10 ppm and the proposed dietary intake as less than 1.5–15 mg per person per day.[14] Therefore, it is highly desirable to develop effective tools for quantitative detection of palladium in the ecological environment. Several methods have been used by various research groups to detect the traces of palladium such as atomic absorption spectrometry, plasma emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS) and solid-phase microextraction-high performance liquid chromatography.[15-19] But all these methods have several disadvantages including slow response, complex process, high consumption of time and high cost.[20-22] Fluorescent technique, on the other hand, provides an efficient method for the detection of very low quantity of palladium and hence, serves as better alternative with various advantages such as low cost, fast response and simple process.[23-32]

A vast variety of fluorescent probes have been developed to detect palladium, based on various fluorophores including naphthalene,[33] acridine,[34] cyanine,[35] N-(3-(benzo[d]thiazol-2-yl)-4-(hydroxyphenyl) benzamide) (HBTBC),[36] triazole,[37] coumarin,[38, 39] rhodamine,[27, 40-49] anthraquinone-imidazole,[50] naphthalimide[51] and fluorescein.[28, 52] The disadvantages of above fluorescent probes include long fluorescence response time,[53, 54] small Stokes shift, [55-57] short emission wavelength[58-61] and fluorescence-quenched response mechanism.[62-71] 2-(2’-hydroxyphenyl)benzothiazole and its derivatives have gained an upsurge of interest due to their ability to undergo an excited-state intramolecular photon transfer (ESIPT) process upon photoexcitation.[72-73] Herein, we have described the synthesis of two 2-(2’-hydroxyphenyl)benzothiazole-based fluorescent probes **1** and **2** (Scheme 1), and their mechanistic aspects regarding the optical changes that lead to emission of strong fluorescence in short duration upon exposure to Pd^{2+}. Owing to the release of 2-(2’-hydroxyphenyl)benzothiazole unit induced by Pd^{2+}, the two probes **1** and **2** exhibited large Stokes shifts with decreased interference of excitation. On the basis of these advantages, probes **1** and **2** exhibited sensitive and efficient potential to detect traces of Pd^{2+}.



1 **2**

Scheme 1. Structures of probes **1** and **2**

2.Experimental

2.1. Materials and methods

2-Aminothiophenol, sodium metabisulfite, TFA and (NH$_4$)$_2$PdCl$_6$ were purchased from Aladdin Co., Ltd.. Pd(AcO)$_2$ was purchased from Sigma-aldrich Co., Ltd.. PdCl$_2$ was purchased from Accela ChemBio Co., Ltd.. 1,3-Indanedione, 3-Bromopropyne and 2-Hydroxy-5-methylbenzaldehyde, were purchased from J&K SCIENTIFIC LTD.. Other reagents of analytical reagent grade and different metal ions were purchased from Sinopharm Chemical Reagent Co., Ltd. and

Sigma-aldrich Co., Ltd.. All reagents and solvents were used without any purification. Deionized water was used throughout all experiments. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Haiyang Chemical Co., Ltd.). ^{1}H NMR and ^{13}C NMR spectra were collected on Bruker AV400 NMR Spectrometer. The chemical shifts in parts-per-million (ppm) for NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference. Mass spectra were obtained from Q-Tof mass spectrometer (Agilent 6530). Fluorescent spectra were measured on RF-5301/PC spectro-fluorophotometer and emission maxima (λ_{max}) were expressed in nanometers. Electronic transition spectra were recorded on α-1860A UV-Vis spectrometer and absorption maxima (λ_{max}) were expressed in nanometers.

2.2. Synthesis

2.2.1. Synthesis of 2-(2'- hydroxyphenyl -5'-methyl)benzothiazole (**3**)

2-Hydroxy-5-methylbenzaldehyde (5 g, 36.72 mmol), 2-aminothiophenol (4.6 g, 36.74 mmol) and sodium metabisulfite (6 g, 31.56 mmol) were dissolve ed in *N, N*-dimethylformamide (DMF) (50 mL) in a round bottom flask. The reaction mixture was refluxed under 110 ^{o}C for 3 h and the progress of reaction was monitored by TLC. After completion of reaction, reactant solution was cooled to room temperature. The solid was precipitated out on addition of deionized water (50 mL) to the solution. The solid was filtered, washed with deionized water for 5 times and dried under vacuum to get the white product **3** (8.5 g, yield 96%). ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 11.36 (1H, s), 8.15 (1H, d, *J* = 8.0 Hz), 8.07 (1H, d, *J* = 8.1 Hz), 7.99 (1H, s), 7.56-7.52 (1H, m), 7.46-7.42 (1H, m), 7.24 (1H, d, *J = 8.3 Hz*), 7.00 (1H, d, *J* = 8.4 *Hz*), 2.33 (3H, s); ^{13}C NMR (DMSO, 100 MHz) δ (ppm): 165.77, 154.66, 151.93, 134.73, 133.71, 128.89, 128.75, 126.92, 125.50, 122.53, 122.46, 118.41, 117.35, 20.50. ESI-MS: calcd for $C_{14}H_{11}NOS$ $[M + H]^+$, 241.0561; found, 241.0655.

2.2.2. Synthesis of 2-(2'- hydroxyphenyl -3'-aldehyde-5'-methyl)benzothiazole (**4**)

Product **3** (2.5 g, 10.37 mmol) and hexamethylenetetramine (HMT) (4.5 g, 32.10 mmol) were dissolved in TFA (15 mL) in a round bottom flask. The reaction mixture was refluxed under 100 ^{o}C for 6 h and the end of reaction was monitored by TLC. After cooling to room temperature, HCl (200 mL, 4 mol/L) was added and the reaction mixture was extracted with dichloromethane, washed with brine and then dried under vacuum. After evaporation of organic solvent, the obtained precipitate was purified by silica gel column chromatography using CH_2Cl_2 as eluent to give faint yellow solid **4** (2 g, yield 72%). ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 12.76 (1H, s), 10.33 (1H, s), 8.22 (1H, d, *J* = 6.6 Hz), 8.20 (1H, s), 8.13 (1H, d, *J* = 8.0 Hz), 7.73 (1H, s), 7.60-7.58 (1H, m), 7.53-7.50 (1H, m), 2.40 (3H, s); ^{13}C NMR (DMSO, 100 MHz) δ (ppm): 192.30, 165.66, 157.51, 151.48, 135.76, 134.04, 133.58, 129.78, 127.42, 126.31, 123.72, 122.78, 122.75, 119.60, 20.21. ESI-MS: calcd for $C_{15}H_{11}NO_2S$ $[M + H]^+$, 269.0510; found, 269.0594.

2.2.3. Synthesis of 2-(2'-(propargyl ether) phenyl -3'-aldehyde-5'-methyl)benzothiazole (**1**)

Product **4** (0.5 g, 1.86 mmol) was dissolved in DMF(50 mL) in a round bottom flask, followed by addition of K_2CO_3 (0.616 g, 4.46 mmol). The reaction mixture was stirred under room temperature for 20 min. Further, 3-bromopropyne (0.531 g, 4.46 mmol) was added to the reaction mixture and stirred for 15 h at room temperature. After completion of reaction as checked by TLC, deionized water was added. The reaction mixture was then extracted with ethyl acetate, washed with brine and dried under vacuum. The crude product obtained was purified by silica column chromatography using dichloromethane–hexane as eluent (2:3, v/v) to give white solid **1** (0.4 g, yield 29%). ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 10.34 (1H, s), 8.47 (1H, s), 8.20 (1H, d, *J* = 7.8 Hz), 8.13 (1H, d, *J* = 8.0 Hz), 7.82 (1H, s), 7.58-7.57 (1H, m), 7.52-7.50 (1H, m), 4.93 (2H, s), 3.65 (1H, s), 2.48 (3H, s); ^{13}C NMR (DMSO, 100 MHz) δ (ppm): 189.99, 161.85, 155.83, 152.37, 136.20, 136.08, 135.99, 132.50, 131.04, 128.48, 127.25, 126.32, 123.58, 122.79, 81.67, 78.60, 64.64, 20.86. ESI-MS: calcd for $C_{18}H_{13}NO_2S$ $[M + H]^+$, 307.0667; found, 307.0710.

2.2.4. Synthesis of 2-(2'-(propargyl ether) phenyl -3'- Indanedione -5'-methyl)benzothiazole (**2**)

Product 1 (0.307 g, 1 mmol) and 1,3-Indanedione (0.161 g, 1.1 mmol) were dissolved in THF (20 mL) in a round bottom flask, followed by addition of 2-3 drops of piperidine. The reaction mixture was stirred for 6 h at room temperature. After completion of reaction as checked by TLC, deionized water was added. The reaction mixture was then extracted with dichloromethane, washed with brine and dried under vacuum. The crude product obtained was purified by silica column chromatography using dichloromethane–hexane as eluent (2:1, v/v) to give yellow solid **2** (0.12 g, 25%). ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 8.61 (1H, s), 8.39 (1H, s), 8.20 (1H, d, *J* = 7.4 Hz), 8.12 (2H, d, *J* = 7.9 Hz), 8.03 (4H, d, *J* = 10.8 Hz), 7.58-7.56 (1H, m), 7.51-7.49 (1H, m), 4.77 (2H, s), 3.59 (1H, s), 2.50 (3H, s); ^{13}C NMR (DMSO, 400 MHz) δ (ppm): 189.27, 188.44, 155.06, 152.73, 152.40, 142.56, 140.23, 138.44, 136.18, 136.07, 134.74, 134.11, 130.98, 129.82, 127.78, 127.46, 126.61, 125.68, 123.34, 123.19, 123.10, 122.11, 79.64, 78.04, 64.17, 20.31. ESI-MS: calcd for $C_{27}H_{17}NO_3S$ $[M + H]^+$, 435.0929; found, 435.0942.

2.3. Absorption and fluorescence measurements

The probe **1** (1 mM) was dissolved in DMSO and maintained at room temperature. Stock solutions (20 mM) of metal ions including Pd^{2+}, Pb^{2+}, Cr^{3+}, Ag^+, Cu^{2+}, Li^+, Fe^{2+}, Zn^{2+}, Eu^{3+}, Co^{3+}, Hg^{2+}, Zr^{4+}, Cs^{2+}, K^+, Na^+, Cd^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, Ca^{2+} were prepared in deionized water by dissolving the corresponding salts. Test solutions were prepared by placing 30 μL of the probe stock solution into a test tube, diluting the solution to 3 mL with HEPES buffer (10 mM, pH = 7.4) and then different analytes were added. All UV-vis absorption and fluorescence measurements were taken at room temperature. Excitation and emission wavelengths were chosen at 380 nm and 543 nm, respectively. The excitation slit width was 10 nm and emission slit width was 5 nm. The fluorescence spectra were recorded after 5 min of addition of analytes into the test tube, to allow complete mixing of analytes into the solution.

It was found that **2** reacted very slowly when exposed to Pd^{2+} in 100% water at room temperature. Hence, different solvent ratios were explored to optimize the reactivity of **2** with Pd^{2+}. It was observed that **2** reacted with Pd^{2+} at different ratios of H_2O–THF at room temperature and exhibited the desired fluorescence. The ratio of H_2O–THF: 2:5 was found to be the best suitable ratio for getting maximum fluorescence (Fig. S1) and hence, prompted us to carry out the response detection of **2** to Pd^{2+} at this ratio.

The probe **2** (1 mM) was dissolved in DMF and maintained at 37 ^oC. Stock solutions (20 mM) of metal ions including Pd^{2+}, Pb^{2+}, Cr^{3+}, Ag^+, Cu^{2+}, Li^+, Fe^{2+}, Zn^{2+}, Eu^{3+}, Co^{3+}, Hg^{2+}, Zr^{4+}, Cs^{2+}, K^+, Na^+, Cd^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, and Ca^{2+} were prepared in deionized water by dissolving the corresponding salts. Test solutions were prepared by placing 30 μL of the probe stock solution into a test tube, diluting the solution to 3 mL with THF-HEPES buffer (10 mM, pH =7.4, 5:2 v/v) and then different analytes were added. All UV-vis absorption and fluorescence measurements were recorded after probe **2** reacted with analytes at 37 ^oC. Excitation and emission wavelengths were chosen at 437 nm and 642 nm, respectively. Both the excitation and emission slit widths were set to 5 nm. After the analytes were added into the test tube, it took 20 min for complete mixing into the solution before carrying out the fluorescence spectral analysis.

2.4. Time-dependent fluorescence studies of probes **1** and **2**

Stock solutions of probe **1** (1 mM) and $PdCl_2$ (0.8 mM) were prepared in DMSO and deionized water, respectively , and were stored at 4 ^oC. Test solution was prepared by placing 30 μL of probe stock solution into the test tube, diluting the solution to 3 mL with HEPES buffer (10 mM, pH =7.4) and adding 30 μL of analyte stock. Excitation and emission wavelengths were chosen at 380 nm and 543 nm, respectively. The excitation slit width was 10 nm and emission slit width was 5 nm.

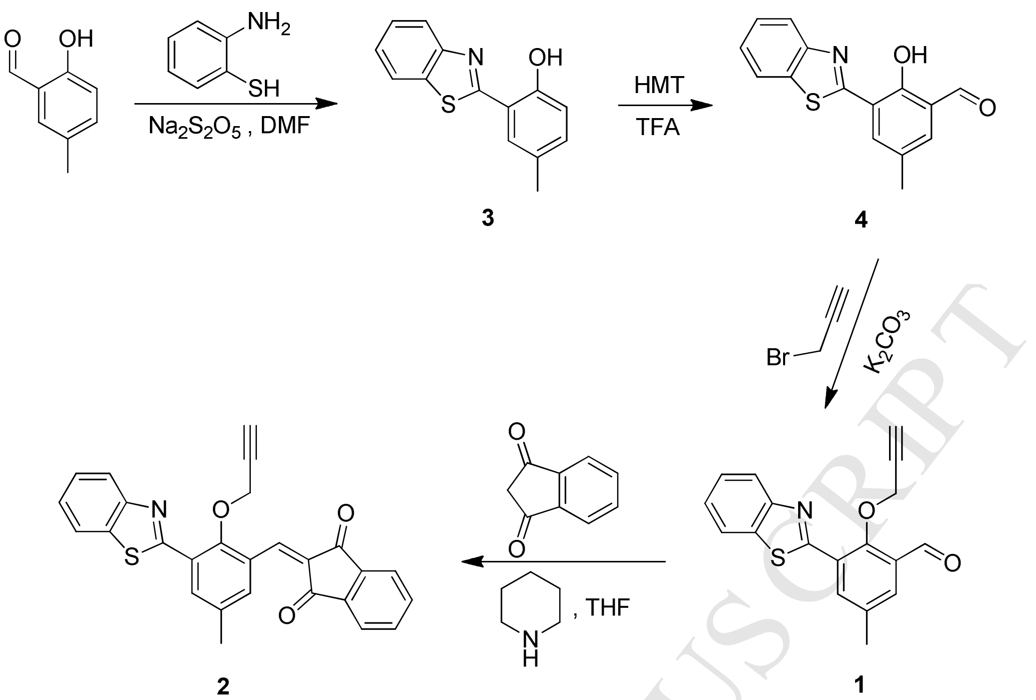
Stock solution of probe **2** in DMF (1 mM) and $PdCl_2$ in deionized water (2 mM) and was stored at 4 ^oC. Test solution was prepared by placing 30 μL of probe stock solution into the test tube, diluting the solution to 3 mL with THF-HEPES buffer (10 mM, pH =7.4, 5:2 v/v) and adding 30 μL of analyte stock. Excitation and emission wavelengths were chosen at 437 nm and 642 nm, respectively. Both the excitation and emission slit widths were 5 nm. Each set of data was measured at regular intervals of 5 min at 37 ^{o}C.

2.5. Preparation of paper test strips loaded with probes **1** and **2**

A volume of 30 μL stock solution of probes **1** and **2** (1 mM) were dropped on the filter papers having diameter of 1 cm, and then test papers were dried in air to measure different concentrations of Pd^{2+}.

3. Results and discussion

The synthetic process of probe **1**, **2** were shown in Scheme 2. Compound **3**, formed by 2-hydroxy-5-methylbenzaldehyde and 2-aminothiophenol, reacted with HMT to give the compound **4**. In alkaline conditions, compound **4** was converted into probe **1**. The probe **2** was obtained through the reaction between aldehyde group and 1,3-indanedione. After purification, two probes were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectroscopy (Fig. S2 - Fig. S13).



Scheme 2. Synthesis of probes **1** and **2**

3.1. Optical responses of probes **1** and **2** to Pd^{2+}

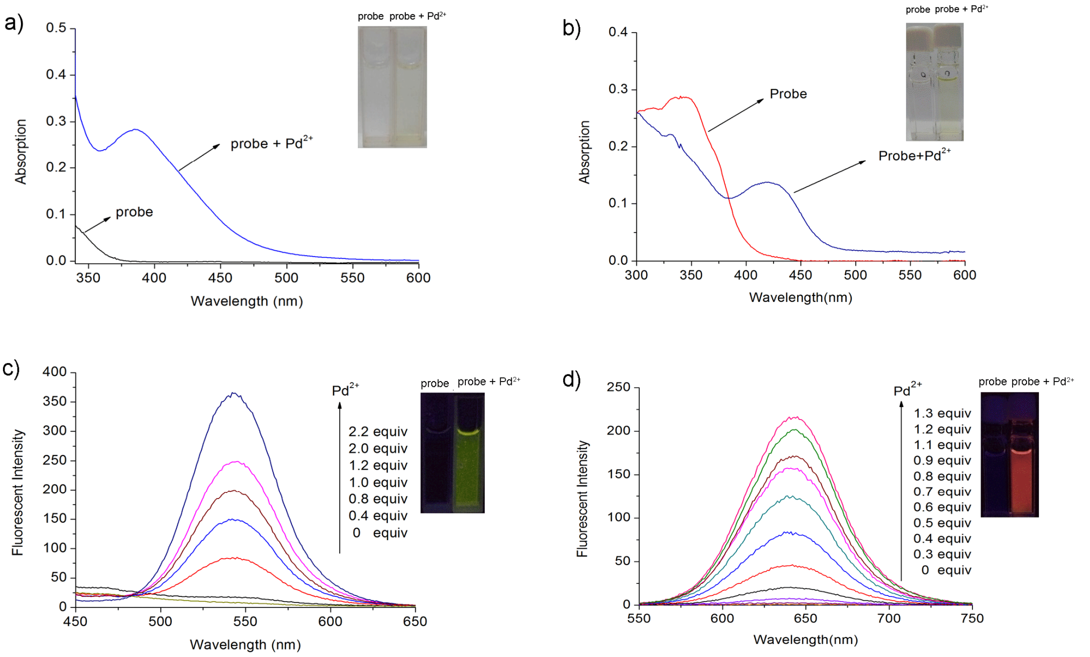


Fig. 1. (a) Absorption spectra of **1** (10 μM) in the absence or presence of Pd^{2+} (22 μM). (b) Absorption spectra of **2** (10 μM) in the absence or presence of Pd^{2+} (13 μM). (c) Fluorescence spectra of **1** (10 μM) upon addition of different concentrations of Pd^{2+} (0 – 22 μM) at room temperature for 5 min. (10 mM HEPES,

pH 7.4, λ_{ex} = 380 nm). (d) Fluorescence spectra of **2** (10 μM) upon addition of different concentrations of Pd^{2+} (0 – 13 μM) at 37 ^{o}C in H_2O-THF (2:5) for 20 min. (10 mM HEPES, pH 7.4, λ_{ex} = 437 nm)

The color and absorption spectral changes of **1** and **2** induced by Pd^{2+} were first examined. As shown in Fig. 1a and Fig. 1b, in the absence of Pd^{2+}, the solution containing **1** or **2** is colorless. With the addition of Pd^{2+}, probe **1** and **2** exhibited different absorption at 380 nm and 425 nm respectively. The remarkable color change from colorless to yellow was clearly visible. The fluorescence responses of probes **1** and **2** to Pd^{2+} were also investigated in detail. When probe **1** was excited at 380 nm upon the addition of Pd^{2+}, the emission peak was observed to be at 543 nm with green fluorescence, along with a Stokes shift of about 163 nm (Fig. 1c). In contrast, probe **2** upon excitation at 437 nm, showed the emission peak at 642 nm in presence of Pd^{2+} with a large Stokes shift of about 217 nm and displayed red fluorescence (Fig. 1d). Both the probes were examined in the absence of Pd^{2+} and resulted in almost no fluorescence. While, remarkable enhancement in their fluorescence intensities were observed in the presence of different concentrations of Pd^{2+}. When the concentration of Pd^{2+} was increased to 1.3 equiv of probe concentration, the fluorescence enhancement of probe **2** reached a plateau of upto 248-fold enhancement, while the fluorescence of probe **1** was found to be increased up to 53-fold at 2.2 equiv. concentration of Pd^{2+}. The two kinds of good fitted regression lines of two probes were shown in Fig.S14 and Fig.S15 and the detection limits of **1** and **2** to Pd^{2+} were calculated as 285 nM and 14.6 nM respectively. This result confirmed that probe **2** is more sensitive to Pd^{2+} than probe **1**.

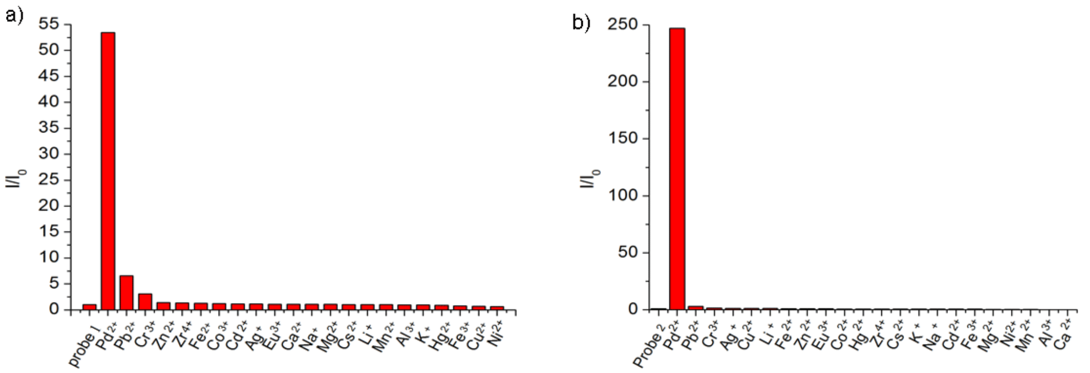


Fig. 2. (a) Fluorescence response of **1** (10 μM) upon addition of different metal ions (200 μM) and Pd^{2+} (100 μM) at room temperature for 5 min in HEPES buffer (10 mM, pH 7.4), $\lambda_{ex} = 380$ nm, $\lambda_{em} = 543$ nm (b) Fluorescence response of **2** (10 μM) upon addition of different metal ions (200 μM) and Pd^{2+} (100 μM) at 37 °C for 20 min in HEPES (10 mM, pH 7.4)-THF (2:5, v/v), $\lambda_{ex} = 437$ nm, $\lambda_{em} = 642$ nm.

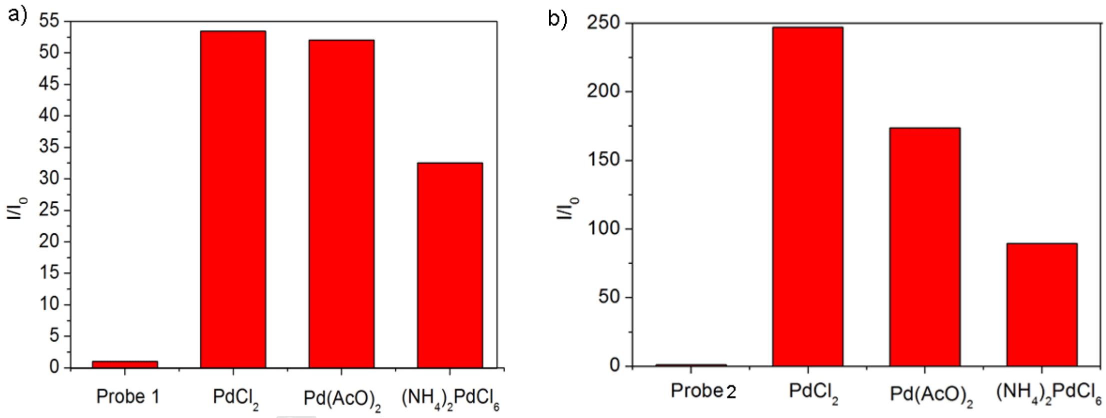
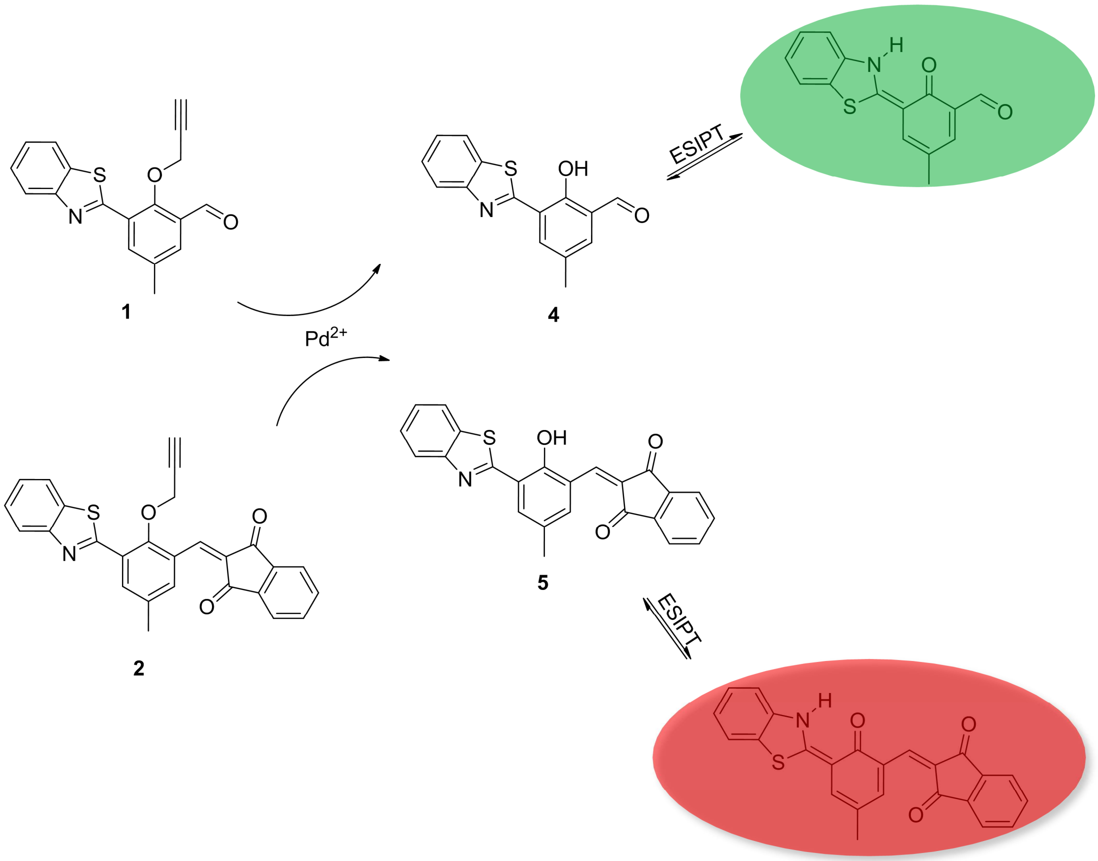


Fig.3. (a) The ratio (I/I_0) of fluorescence spectra of probe **1** (10 μM) upon adding different species of palladium (110 μM) for 5 min at 543nm in HEPES buffer (10 mM, pH 7.4), $\lambda_{ex} = 380$ nm. (b) The ratio (I/I_0) of fluorescence spectra of probe **2** (10 μM) at 642 nm upon adding different species of palladium (110 μM) at 37 °C for 20 min in HEPES (10 mM, pH 7.4)-THF (2:5, v/v), $\lambda_{ex} = 437$ nm.

Further, the selectivity of probe **1** and **2** was studied by examining the responses of probes to different metal ions including Pd^{2+}, Pb^{2+}, Cr^{3+}, Ag^{+}, Cu^{2+}, Li^{+}, Fe^{2+}, Zn^{2+}, Eu^{3+}, Co^{3+}, Hg^{2+}, Zr^{4+}, Cs^{2+}, K^{+}, Na^{+}, Cd^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Al^{3+}, Ca^{2+}.

Under similar test conditions of probe **1** or probe **2**, all metal ions (20 equiv.) except for Pd^{2+} shown rarely fluorescent changes, only Pd^{2+} could give significant rise in fluorescent intensity (Fig. 2). As the figure indicated, probes **1** and **2** were having excellent detection capability to Pd^{2+}. Pd^{2+} was easily differentiated among various metal ions due to emission of green fluorescence and red fluorescence using handhold UV lamp (Fig. S16). No drastic changes in fluorescence response of probe **1** and **2** were noticed at different pH levels, and hence, probe **1** and **2** can be applied in acidic and alkaline conditions (Fig. S17). Considering that palladium not only exists in the form of palladium chloride, so we tested the fluorescence response of two probes to another two species of palladium ($(NH_4)_2PdCl_6$, $Pd(AcO)_2$). Both probe **1** and probe **2** were observed to produce significant fluorescence enhancement on reacting with $PdCl_2$, $(NH_4)_2PdCl_6$, and $Pd(AcO)_2$. Hence, it is confirmed that these two probes can determine various palladium species effectively (Fig. 3). The responses of **1** and **2** to Pd^{2+} were found to be very rapid. As shown in Fig. S18, in the presence of Pd^{2+}, both the solutions containing probes **1** and **2** displayed maximum fluorescent intensity within 10 minutes.

3.2. Proposed mechanism



Scheme 3. Proposed signaling mechanism of probe **1**, **2** to Pd^{2+}

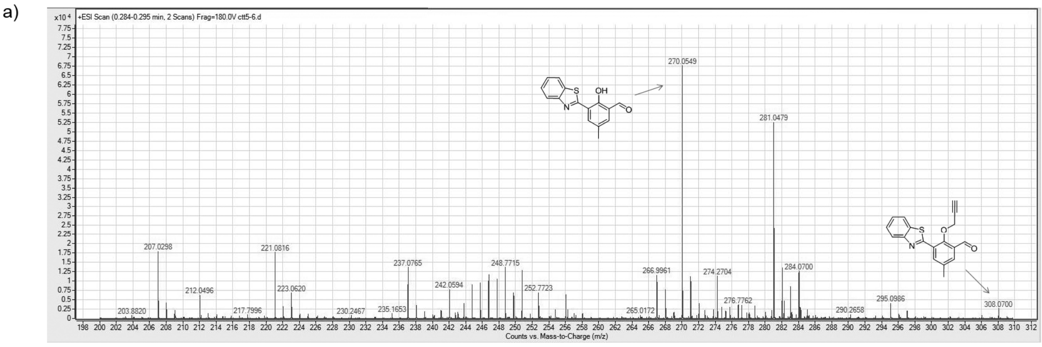


Fig. 4. ESI-MS spectra of mixture of probe **1** (a) and **2** (b) in the presence of Pd^{2+}.

Furthermore, we proposed the possible detection mechanism for optical changes induced by Pd^{2+}. The optical changes should be attributed to the cleavage of alkynyl ether linkage induced by Pd^{2+}, which released the moieties **4** and **5**, under light irradiation, **4** and **5** converted to the corresponding ketones structure and leading to strong fluorescence (Scheme 3). In order to verify our hypothesis, the ESI-MS analysis of the solution of probes reacted with Pd^{2+} were carried out. As shown in Fig. 4(a) and 4(b), two new signals at m/z = 270.0549 and 397.0708 appeared after addition of Pd^{2+}, which were assigned to mass peaks of **4** and **5**, demonstrated that Pd^{2+} was responsible to induce the cleavage of alkynyl ether linkage.

3.3. Analytical Application

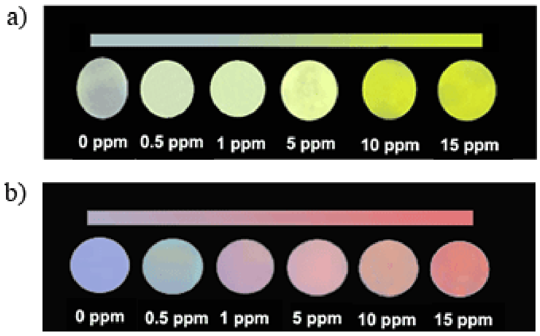


Fig. 5. The photographs of probe **1**(a) and **2**(b) loaded test strips, treated with different concentrations of Pd^{2+} for 5 min at room temperature, taken under 365 nm UV light.

Considering **1** and **2** have good selectivity to Pd^{2+}, we decided to put the two probes for practical application. Test papers, made from stock solution of two probes were exposed to different concentrations of Pd^{2+}, and then fluorescence changes were observed directly using 365 nm handheld UV lamp. As shown in **Fig. 5**, almost no fluorescence was observed with **1** and **2** loaded on test strips. With increasing Pd^{2+} concentrations, green and red fluorescence of **1** and **2** were enhanced gradually. This confirmed that lowest possible ppm levels of Pd^{2+} can be detected easily by the test strips. Therefore, **1** and **2** can be successfully used as portable fluorescent probes for Pd^{2+}.

4. Conclusions

In conclusion, highly sensitive and selective ESIPT based fluorescent probes **1** and **2** were synthesized for the detection of traces of Pd^{2+}. These probes produced green and red fluorescence along with the large Stokes shifts. The detection limit was found to be as low as 285 nM and 14.6 nM respectively. The portable test strips were prepared by direct deposition of probe molecules onto the filter paper. Thus, our designed probes act as valuable fluorescent probes for quantitative determination of Pd^{2+}.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

Supporting information for "Highly sensitive and selective ESIPT-based fluorescent probes for detection of Pd^{2+} with large Stocks shifts"

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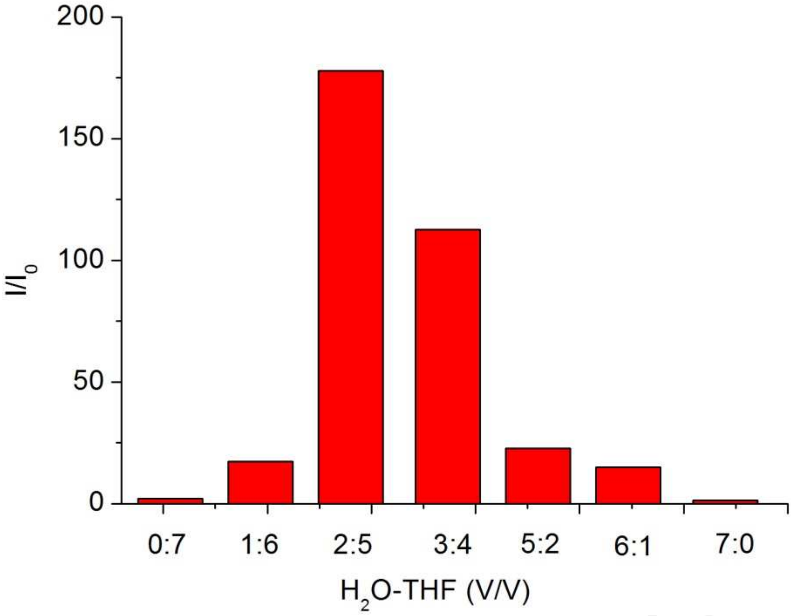


Fig.S1. Fluorescence enhanced factor (I/I$_0$) of **2** (10 μM) in the presence of Pd^{2+} (100 μM) in H_2O-THF at various ratios with excitation at 437 nm.

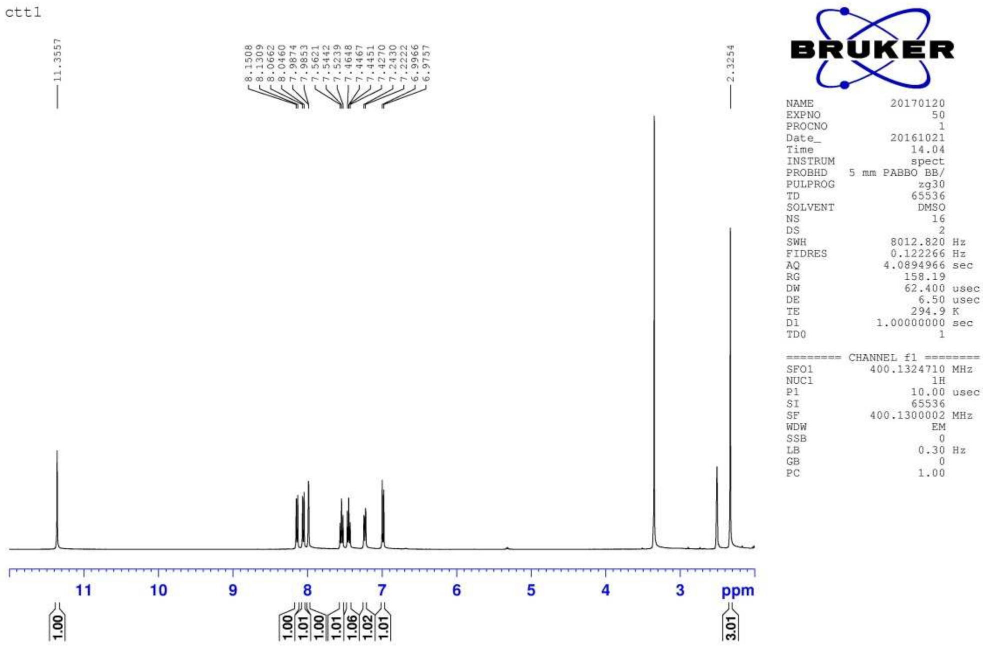


Fig.S2. ^{1}HNMR spectrum of **3** in DMSO.

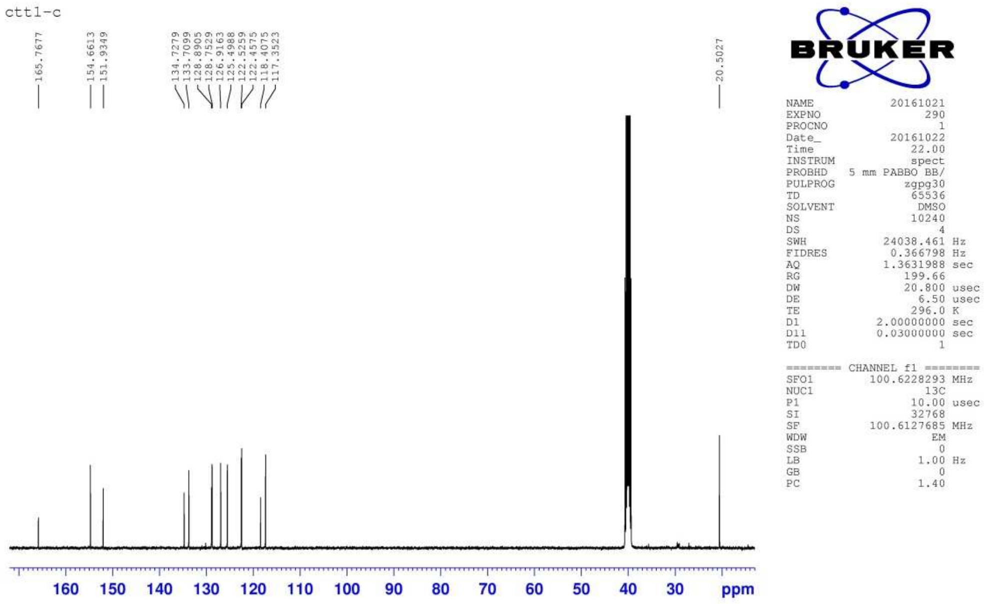


Fig.S3. ^{13}CNMR spectrum of **3** in DMSO.

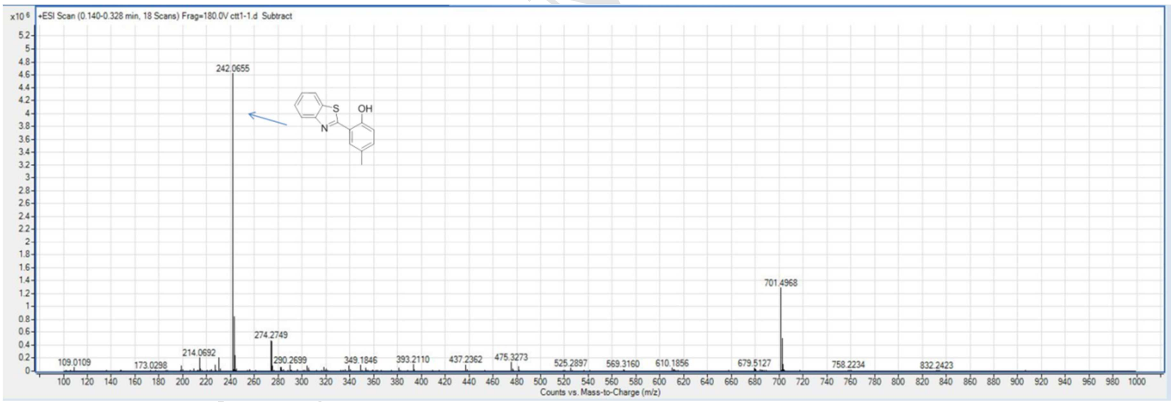


Fig.S4. ESI mass spectrum of **3**.

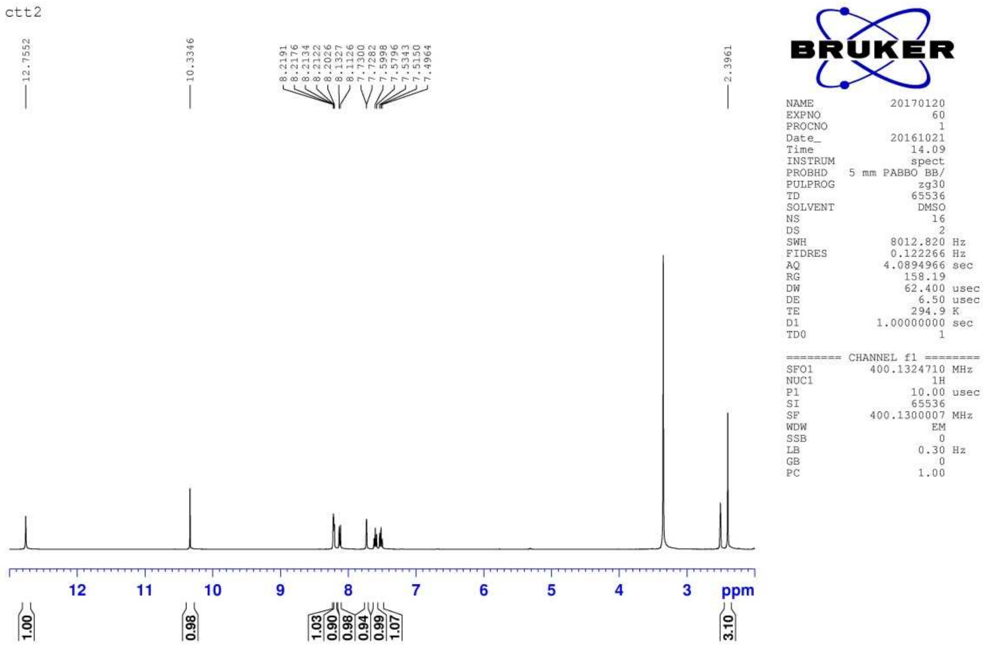


Fig.S5. ^{1}HNMR spectrum of **4** in DMSO.

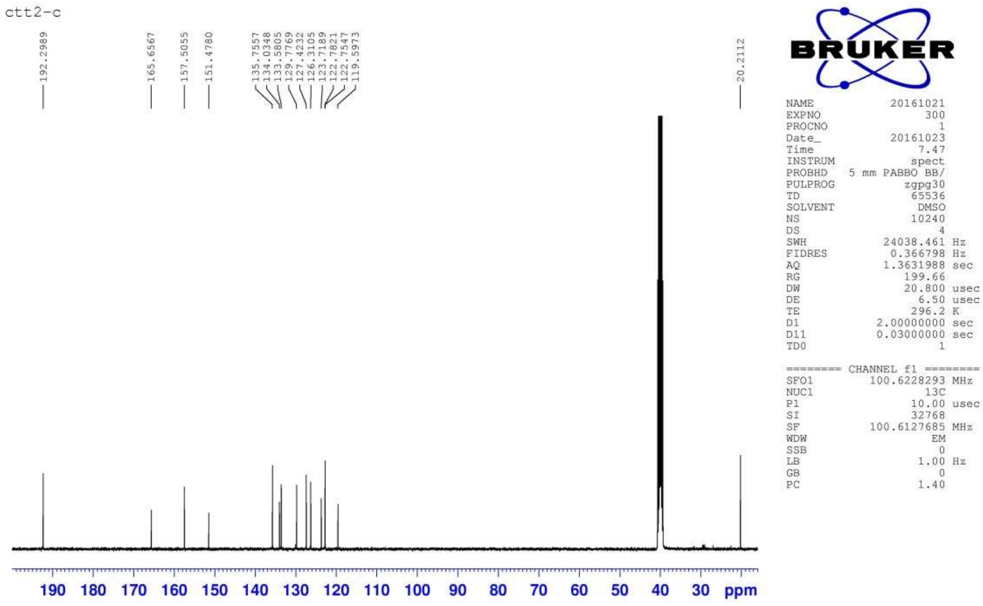


Fig.S6. ^{13}CNMR spectrum of **4** in DMSO.

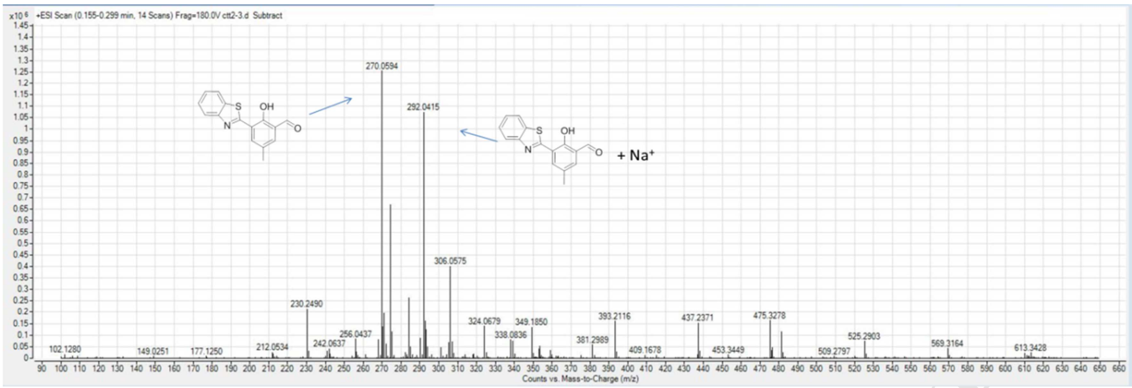


Fig.S7. ESI mass spectrum of **4**.

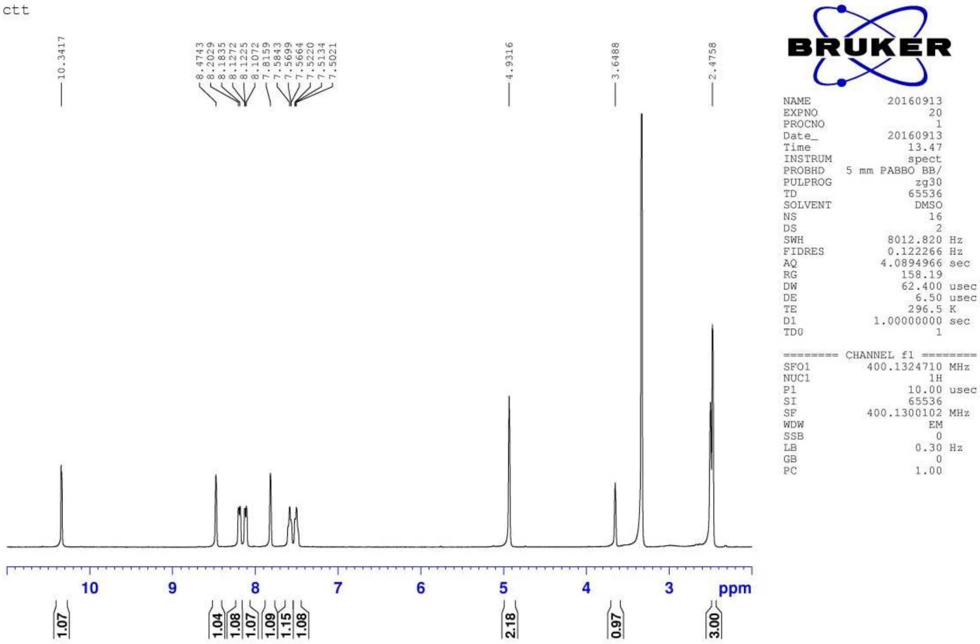


Fig.S8. ^{1}HNMR spectrum of **1** in DMSO.

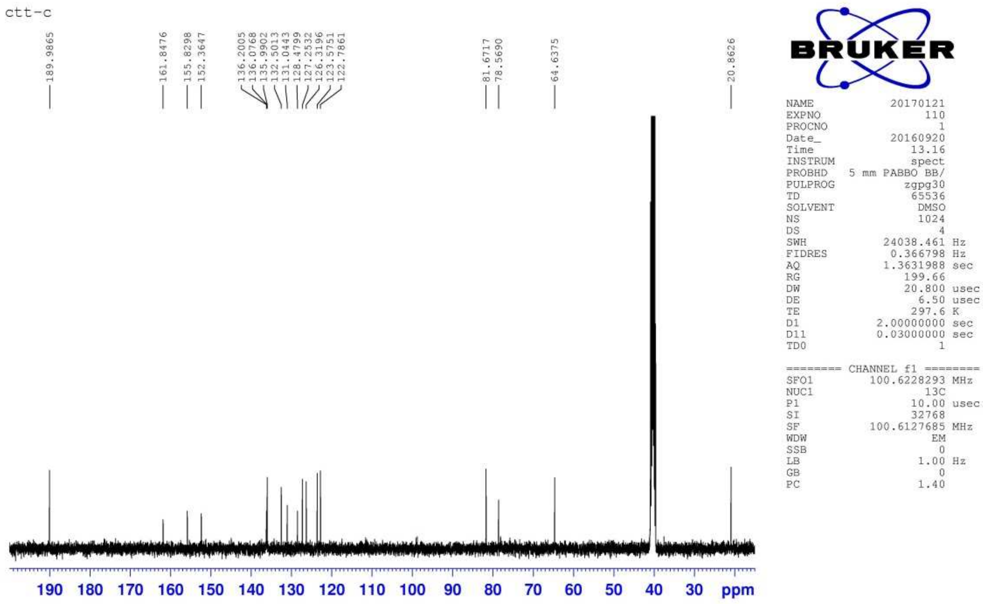


Fig.S9. ^{13}CNMR spectrum of **1** in DMSO.

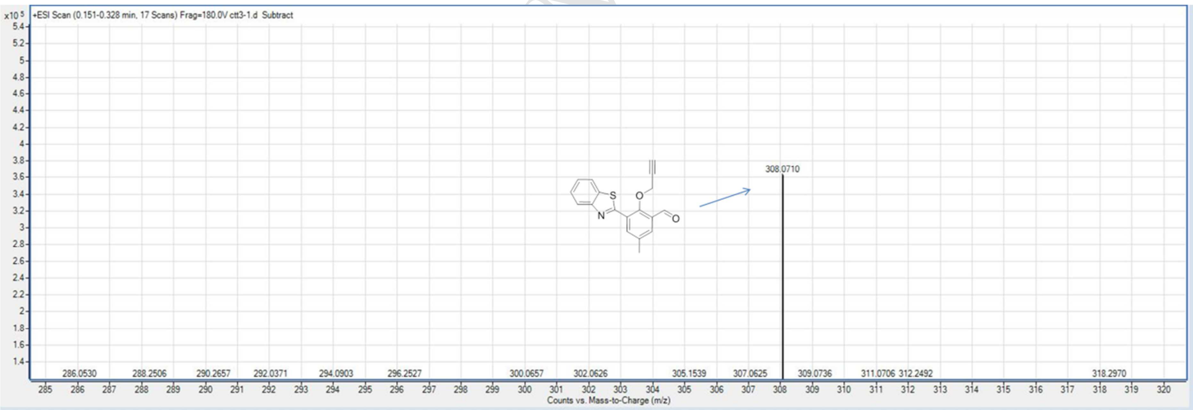


Fig.S10. ESI mass spectrum of **1**.

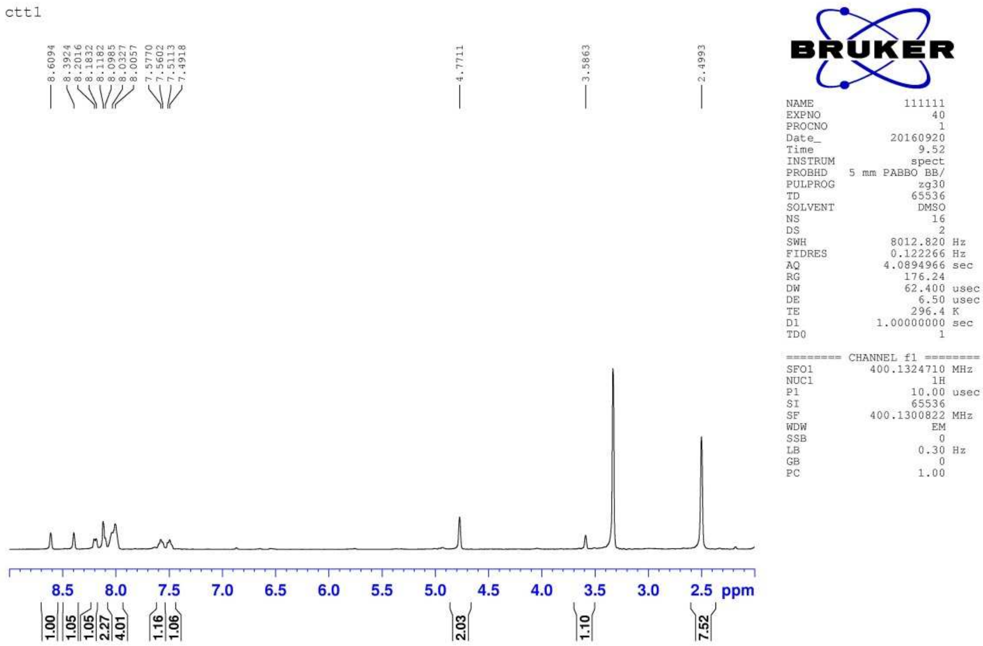


Fig.S11. ^{13}HNMR spectrum of **2** in DMSO.

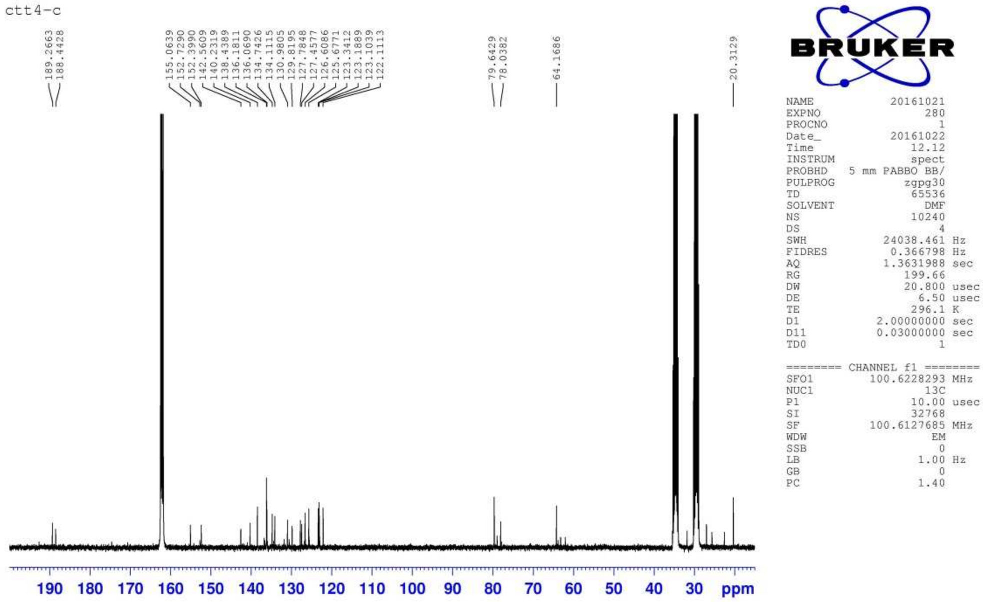


Fig.S12. ^{13}CNMR spectrum of **2** in DMF.

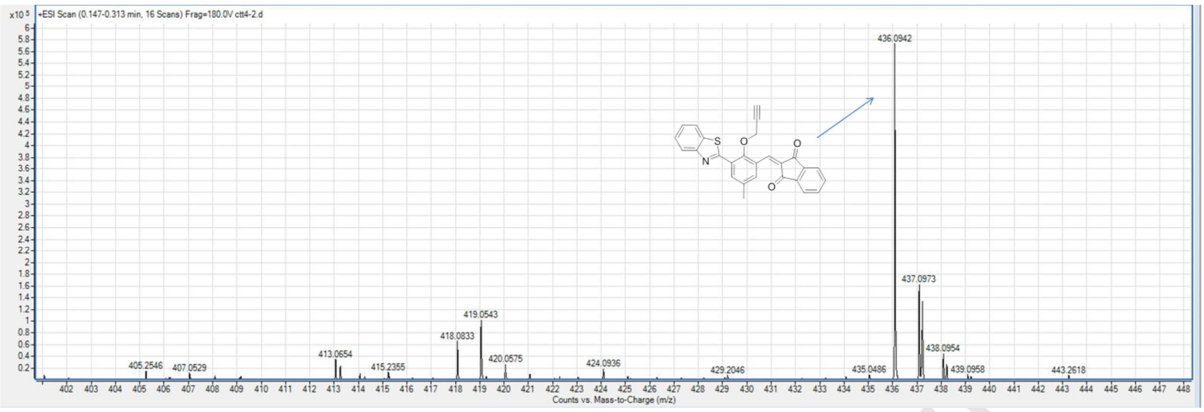


Fig.S13. ESI mass spectrum of **2**.

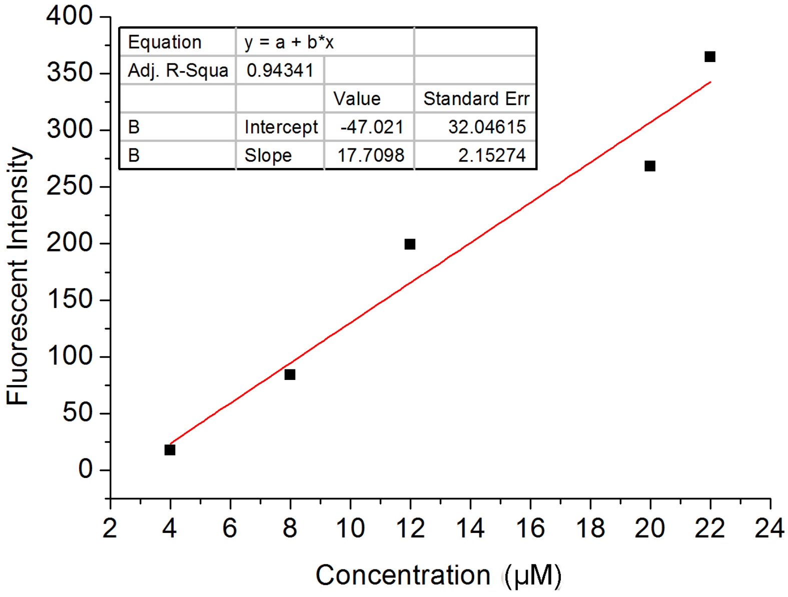


Fig.S14. the linear relationship of **1** between fluorescence intensity at 543 nm and Pd^{2+} concentration. (λ_{ex} = 380 nm, slit: 10 nm/5nm)

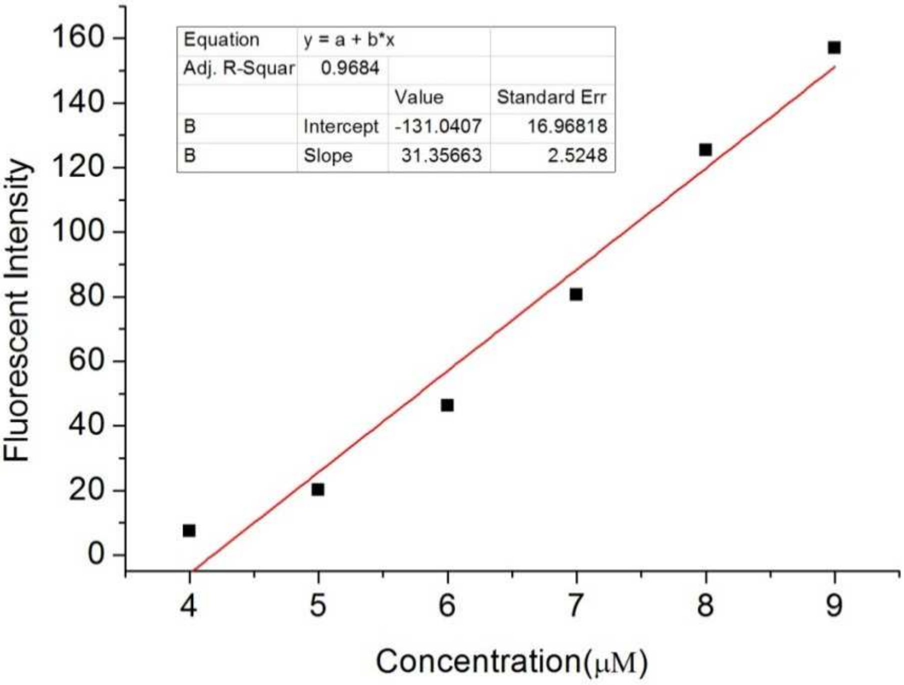
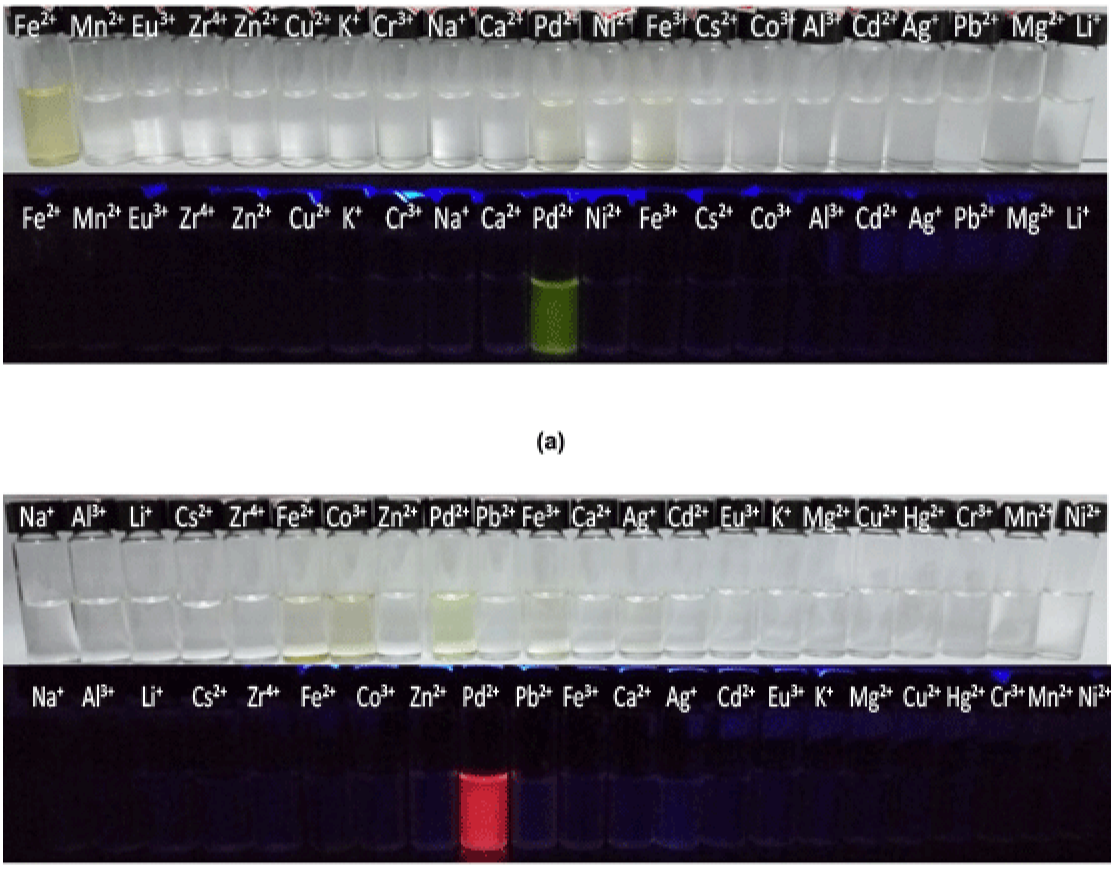


Fig.S15. the linear relationship of **2** between fluorescence intensity at 642 nm and Pd^{2+} concentration. (λ_{ex} = 437 nm, slit: 5 nm/5 nm)



(a)

(b)

Fig.S16. (a) Photographs of probe **1** in the presence of Fe^{2+}, Mn^{2+}, Eu^{3+}, Zr^{4+}, Zn^{2+}, Cu^{2+}, K^+ , Cr^{3+}, Na^+, Ca^{2+}, Pd^{2+}, Ni^{2+}, Fe^{3+}, Cs^{2+}, Co^{3+}, Al^{3+}, Cd^{2+}, Ag^+, Pb^{2+}, Mg^{2+}, Li^+ and (b) **2** (10 μM) in the presence of Na^+, Al^{3+}, Li^+, Cs^{2+}, Zr^{4+}, Fe^{2+}, Co^{3+}, Zn^{2+}, Pd^{2+}, Pb^{2+}, Fe^{3+}, Ca^{2+}, Ag^+, Cd^{2+}, Eu^{3+}, K^+ , Mg^{2+}, Cu^{2+}, Hg^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+} under white light and a 365 nm UV lamp.

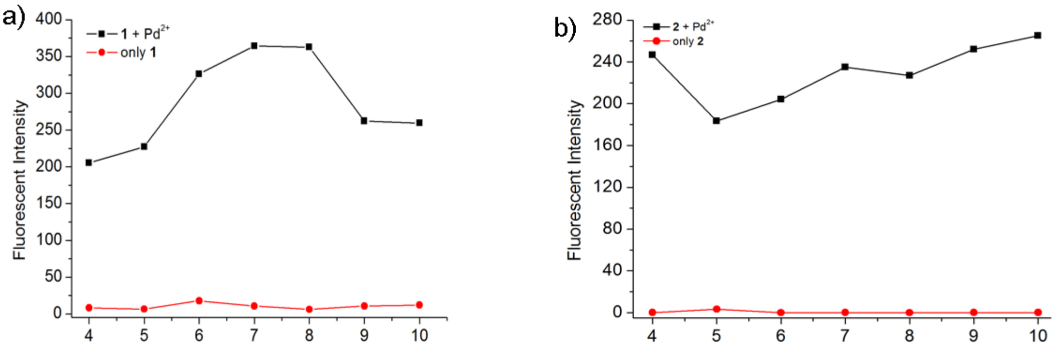


Fig. S17. The responses of probe **1** (10 μM) and **2** (10 μM) to Pd^{2+} at different pH conditions.

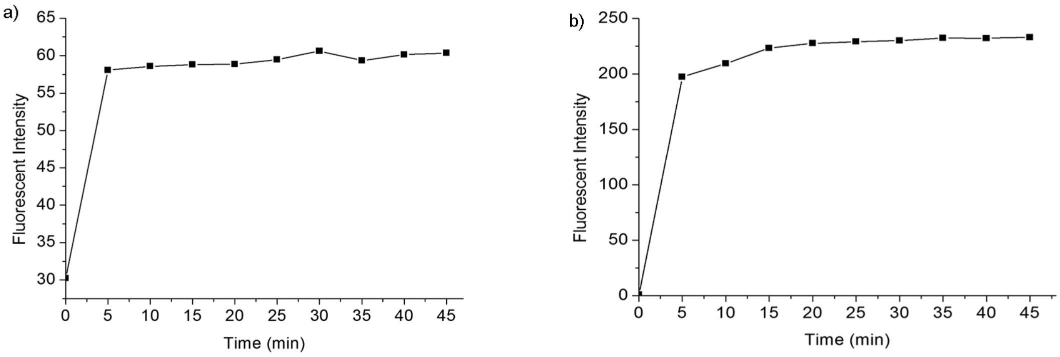


Fig.S18. (a) Time-dependent fluorescence intensity of **1** (10 µM) at 543 nm in the presence of Pd^{2+} (8 µM) (λ_{ex}= 380 nm, slit: 10 nm/ 5 nm). (b) Time-dependent fluorescence intensity of **2** (10 µM) at 642 nm in the presence of Pd^{2+} (20 µM) at 37 ^{o}C in H_2O-THF (2:5) (λ_{ex}= 437 nm, slit: 5 nm/ 5 nm).

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Highlights

1. ESIPT-based fluorescent probes with large Stocks shifts are developed for detection of Pd^{2+}.
2. The probes exhibited good selectivity to Pd^{2+} among various metal ions.
3. The high sensitivity of probes 1 and 2 with detection limits of 285 nM and 15 nM were determined, respectively.
4. The portable test strips prepared by direct deposition of the probe molecules onto the filter paper can detect the lowest possible ppm levels of Pd^{2+}.