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Electronic Supplementary Information

for

A new fluorogenic probe for the selective detection of carbon monoxide in aqueous medium based on Pd(0) mediated reaction

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Experimental section:

1. Materials and physical methods: All reagents and chemicals were purchased from Sigma and used without further purification. Solvents used for spectroscopic studies were purified and dried by standard procedures before use. Fluorescence spectra and absorption spectra were performed using a HITACHI F-4500 Fluorescence Spectrophotometer and JASCO UV/VIS/NIR V-570 Spectrophotometer respectively. Electron spray ionization (ESI) mass spectra were recorded on a Qtof Micro YA263 mass spectrometer. NMR spectra of organic compounds were obtained on a Bruker Advance DPX 400.

2. HPLC analyses: Reversed-phase HPLC analysis was performed with an Inertsil ODS3 column. HPLC separation was carried out with an increasing ratio of buffer B (0.1% HCOOH in acetonitrile) to buffer A (0.1% HCOOH in H_2O). All samples were evaluated by increasing the amount of buffer B from 20 to 90% over 20 min.

3. General method of fluorescence analysis and quantum yield calculation: PCO-1 was dissolved in DMSO to obtain stock solutions for fluorometric titration study. Desired volume of DMSO stock was taken to dilute in 10 mM HEPES buffer (0.4 % DMSO) of pH 8.0 at 37 °C to reach the final concentration (10 μM) of the probe. The fluorescence spectra of PCO-1 were recorded at an excitation wavelength of 340 nm. Relative fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves with the equation:[1]

$$\Phi_{sample} = \Phi_{standard} \times \frac{OD_{standard} \times A_{sample} \times \eta^2_{sample}}{OD_{sample} \times A_{standard} \times \eta^2_{standard}}$$

where OD is optical density of the compound at the excitation wavelength, A is the area under the fluorescence spectral curve and η is the refractive index of the solvents. The standard used for the measurement of fluorescence quantum yield was anthracene (Φ = 0.36 in cyclohexane, λ_{ex} = 350 nm).[2]

4. Cell culture: A549, human lung carcinoma cell lines were collected from National Centre for Cell Science, Pune, India, and used throughout the study. Cells were cultured in DMEM (Gibco BRL) supplemented with 10% FBS (Gibco BRL), and a 1% antibiotic mixture containing PSN (Gibco BRL) at 37ºC in a humidified incubator with 5% CO_2 and cells were grown up to 80-90% confluence, harvested with 0.025% trypsin (Gibco BRL) and 0.52 mM EDTA (Gibco BRL) in phosphate-buffered saline (PBS), plated at the desired cell concentration and allowed to re-equilibrate for 24 h before any treatment. All experiments were conducted in DMEM containing 10% FBS and 1% PSN antibiotic.

5. Fluorescence imaging study: Cells were rinsed with PBS and incubated with 10 μM PCO-1 in 10 mM HEPES buffer (0.4 % DMSO) of pH 8.0 for 20 min at 37 ºC. Cells were then washed thrice with the same buffer and fixed with 4% PFA for 10 minutes and washed again with buffer. Permeabilization of the cells was done using 0.2% Triton X 100 for 5 minutes. Then A549 cells were incubated with $PdCl_2$ (10 μM) for 15 min. Cells were then washed thrice with buffer and finally 10, 20 and 50 μM of CORM-3 were added and again incubated with 30 min at 37 ºC. After incubation, bright field and fluorescence images of A549 cells were taken by a fluorescence microscope (Model: LEICA DMLS) associated with an objective lens of 20X magnification.

6. Cell cytotoxicity assay: In order to test the cytotoxicity of PCO-1, 3-(4, 5-dimethylthiazol-2-yl)-2,S-diphenyltetrazolium bromide (MTT) assay was performed by the procedure described earlier.[3] After treatment with PCO-1 (5, 10, 20 and 50μM) for 4 h, 10 μl of a MTT solution (10mg/ml PBS) was added in each well of a 96-well culture plate and incubated continuously at 37ºC for 3 h. All media were removed from wells and 100 μl of acidic isopropyl alcohol was added into each well. The intracellular formazan crystals (blue-violet) formed were solubilized with 0.04 N acidic isopropyl alcohol and absorbance of the solution was measured at 595 nm wavelength with a microplate reader (Model: THERMO MULTI SCAN EX). The cell viability was expressed as the optical density ratio of the treatment to control. Values were calculated as mean of three independent experiments. The cell cytotoxicity was calculated as % cell cytotoxicity = 100% - % cell viability.

7. Synthesis of compounds (Scheme S1):

a) Synthesis of 1: A solution of 0.495 g (2.3 mmol) of di-tert-butyl dicarbonate in 30 mL of CH_2Cl_2 was added drop wise to 1.0 g (11.3 mol) of *N,N′*-dimetylethylenediamine at 5 °C. The mixture was stirred at room temperature for 24h and then evaporated. The residue was dissolved in 100 mL of ethylacetate and washed with 100 mL of saturated aqueous Na_2CO_3 and 100 mL of saturated NaCl solution. The organic layer was evaporated in vacuo and the crude product was purified by silica gel chromatography (eluent: A→MeOH+MeCN+NEt_3 (2:2:1), 7% A in dichloromethane) to give 1.322 g (62%) of compound **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H), 2.24 (s, 1H), 2.47 (s, 3H), 2.74-2.77 (t, 2H), 2.88 (s, 3H) and 3.33-3.37 (t, 2H); ESI-MS (m/z) found 189.1624 (calculated for [M+H$^+$] 189.1603).

b) Synthesis of 2: To a suspension of *N,N′*-carbonyldiimidazole (0.356 g, 2.2 mmol) in THF (30 mL) was added the compound **1** (0.360 g, 1.9 mmol). The mixture was refluxed for 17 h. Removal of solvent under vacuum gave viscous oil, which was dissolved in CH_2Cl_2 (100 mL) and washed with water (2 × 100 mL). The organic layer was evaporated in vacuo and the crude product was purified by silica gel chromatography with methanol/dichloromethane (1:24, v/v) as eluent to give 0.496 g (92%) of compound **2**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.85 (s, 3H), 3.17 (s, 3H), 3.51-3.54 (t, 2H), 3.65-3.68 (t, 2H), 7.07 (s, 1H) 7.25 (s, 1H) and 7.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.4, 34.5, 37.4, 45.4, 47.7, 79.9, 118.0, 129.4, 137.0 and 151.8; ESI-MS (m/z) found 283.1782 (Calculated for [M+H$^+$] 283.1770).

c) Synthesis of 3: To a solution of compound **2** (0.564 g, 2.0 mmol) in acetonitrile (15 mL) was added methyl iodide (2.838 g, 20.0 mmol). The mixture was stirred at room temperature for 24 h. The solvent was removed under vacuum to yield compound **3** (0.729 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.90 (s, 3H), 3.31 (s, 3H), 3.52-3.55 (t, 2H), 3.69-3.72 (t, 2H), 4.26 (s, 3H), 7.79 (s, 2H) and 9.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2, 34.8, 37.9, 45.5, 49.1, 79.8, 121.0, 123.9, 136.6, 146.9 and 156.2; ESI-MS (m/z) found 297.1936 (Calculated for [M$^+$] 297.1927).

d) Synthesis of 4: To a solution of **3** (0.848 g, 2.0 mmol) in acetonitrile (20 mL) was added the 7-hydroxycoumarin (0.324 g, 2.0 mmol) and triethylamine (0.351 mL, 2.5 mmol). The reaction was refluxed overnight. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 (30 mL) and 0.1 M HCl (10 mL) was added. The aqueous layer was expected with CH_2Cl_2 (3 ×15 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL). The organic layer was evaporated in vacuo and the crude product was purified by silica gel chromatography with ethyl acetate/hexane (1:1, v/v) as eluent to give 0.316 g (42%) of compound **4**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H), 2.93 (s, 3H), 3.06-3.14 (m, 3H), 3.49-3.58 (m, 4H), 6.36-6.38 (d, 1H), 7.07-7.13 (m, 2H), 7.45-7.48 (m, 1H) and 7.67-7.70 (d, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.4, 35.3, 47.3, 52.8, 90.6, 92.6, 110.2, 112.6, 115.7, 118.5, 128.3, 142.9, 154.6 and 160.4; ESI-MS (m/z) found 377.1721 (Calculated for [M+H$^+$] 377.1713).

e) Synthesis of PCO-1: The protected compound **4** (1.149 g, 3.0 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2:trifluoroacetic acid (TFA) (8 mL) and the solution was stirred for 3 h. The solution was removed in vacuo, and CH_2Cl_2 was successively added and evaporated to remove residual TFA, to provide the deprotected compound. To a stirred solution of the deprotected compound in CH_2Cl_2 (5 mL), 20 mL pyridine was added at –5 °C. Then allyl chloroformate (0.372 mL, 3.5 mmol) was added drop wise and then solution was allowed to stirr for 24 h at room temperature. 20 mL CH_2Cl_2 was added to the reaction mixture and washed several times with 10% citric acid to remove the pyridine. The organic layer was evaporated in vacuo and the crude product was purified by silica gel chromatography with ethyl acetate/hexane (3:2, v/v) as eluent to give 0.253 g (23%) of PCO-1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.99 (s, 3H), 3.04-3.15 (m, 3H), 3.54-3.63 (m, 4H), 4.59-4.62 (d, 2H), 5.19-5.22 (d, 1H), 5.27-5.31 (d, 1H), 5.89-5.98 (m, 1H), 6.36-6.38 (d, 1H), 7.07-7.09 (m, 1H), 7.12 (s, 1H), 7.45-7.47 (d, 1H) and 7.67-7.69 (d, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.7, 45.9, 65.5, 109.4, 114.7, 115.6, 117.0, 118.0, 128.0, 132.4, 142.6, 152.9, 153.5, 153.9, 155.5 and 159.7; ESI-MS (m/z) found 361.1408 (Calculated for [M+H$^+$] 361.1400).

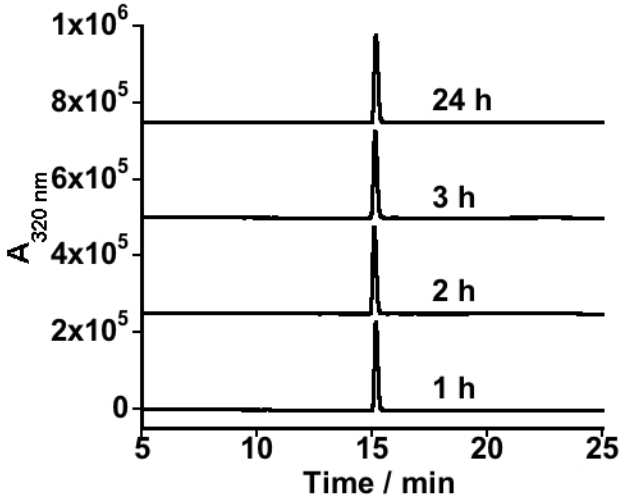


Fig. S1 Reversed-phase HPLC analyses of the stability of the probe, PCO-1 (10 μM). PCO-1 was incubated for the designated period in the reaction buffer [10 mM HEPES, 0.4% DMSO] of pH 8.0 at 37 °C.

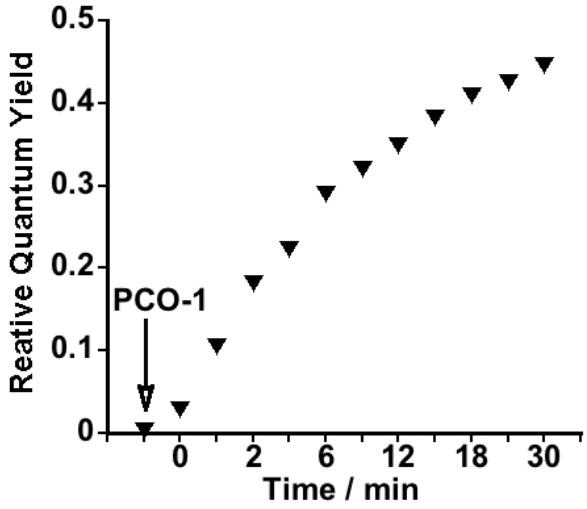


Fig. S2 Plot of relative quantum yield of PCO-1 (10 μM) upon treatment with $Pd(PPh_3)_4$ (10 μM) with the progress of time in reaction of buffer pH 8.0 at 37 °C (ex: 340 nm).

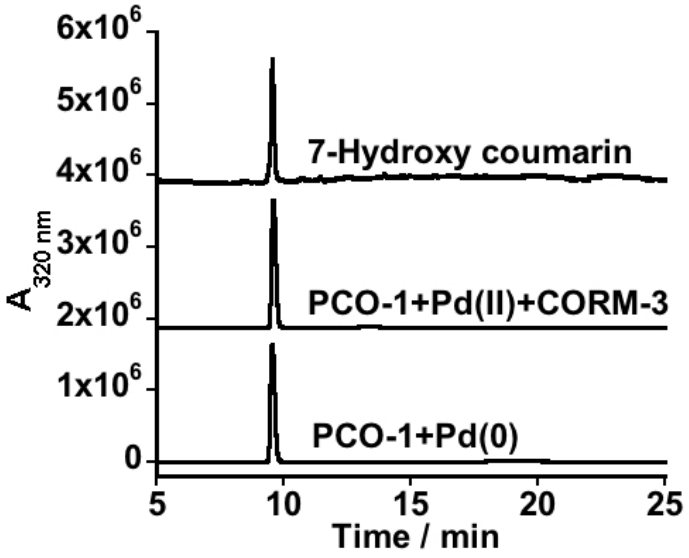


Fig. S3 Reversed-phase HPLC of the reaction progress of PCO-1 with Pd(PPh3)4 and in situ generated Pd(0) by the reaction between $PdCl_2$ and CORM-3 in the first and second row respectively in the reaction buffer incubated for 30 min. Third row referred to the control experiment with 7-hydroxycoumarin.

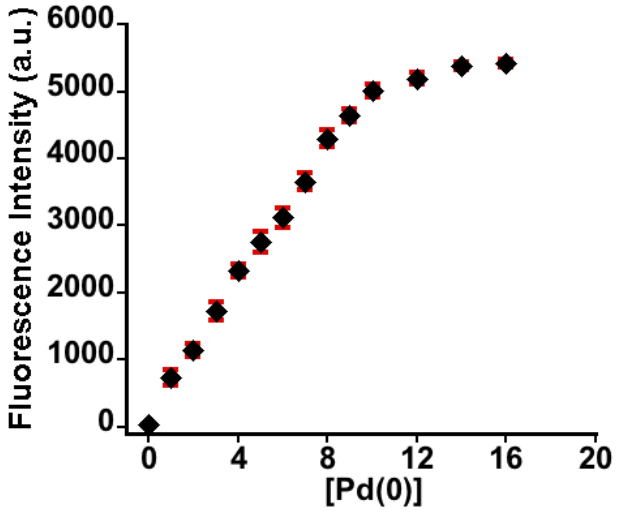


Fig. S4 Fluorescence intensities at 460 nm of PCO-1 (10 μM) with various concentration of $Pd(PPh_3)_4$ (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 μM) were plotted. All sets of experiment were incubated for 30 min in reaction buffer [10 mM HEPES, 0.4% DMSO] of pH 8.0 at 37 °C (ex: 340 nm).

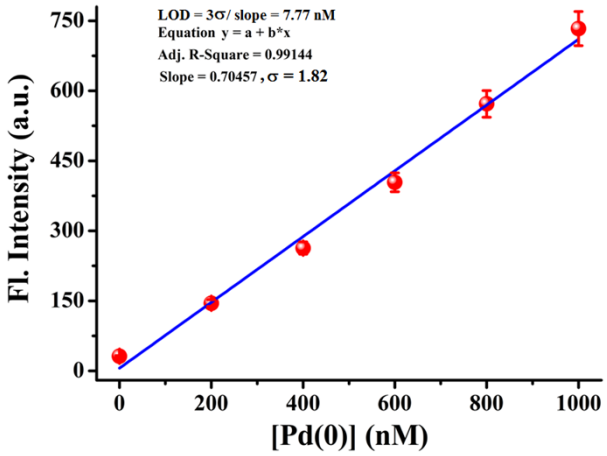


Fig. S5 Plot for the determination of limit of detection (LOD) for Pd(0).

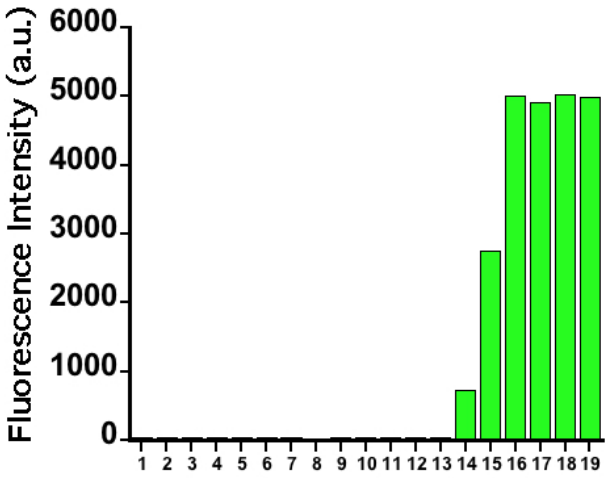


Fig. S6 The fluorescence intensity (460 nm) change profile of PCO-1 (10 μM) in presence of various 3d metal ions incubated for 30 min in reaction buffer of pH 8.0 at 37 °C (ex: 340 nm). [1-none, 2- Cr^{3+}, 3-Mn^{2+}, 4-Fe^{2+}, 5-Fe^{3+}, 6-Co^{2+}, 7-Ni^{2+}, 8-Cu^{2+}, 9-Zn^{2+}, 10-Na^{+}, 11-K^{+}, 12-Ca^{2+}, 13-Mg^{2+}, 14-Pd^{0} (1 μM), 15-Pd^{0} (5 μM), 16-Pd^{0} (10 μM), 17-Pd^{0} (10 μM)+Na^{+}+K^{+}+Ca^{2+}, 18- Pd^{0} (10 μM)+Fe^{2+}+Fe^{3+}+Mn^{2+} and 19-Pd^{0} (10 μM)+Cu^{2+}+Zn^{2+}]. Concentrations of the metal ions except Pd^{0} were used as 100 μM. $Pd(PPh_3)_4$ was used as Pd^{0} source and chlorides salts for the other metal.

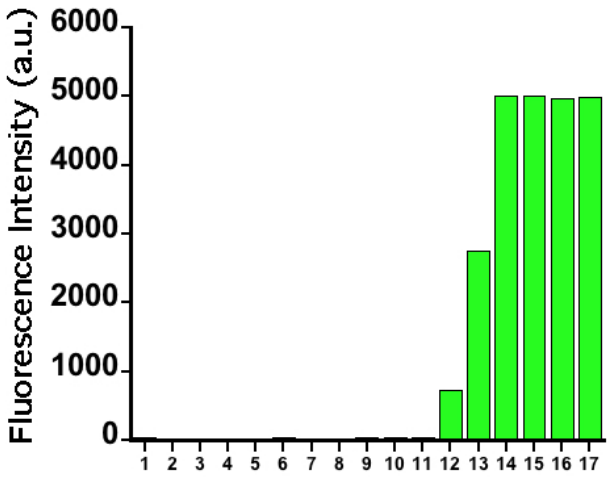


Fig. S7 The fluorescence intensity (460 nm) change profile of PCO-1 (10 μM) in presence of various heavy metal ions incubated for 30 min in reaction buffer of pH 8.0 at 37 °C (ex: 340 nm). [1-none, 2- Ru^{3+}, 3-Hg^{2+}, 4-Cd^{2+}, 5-Ag^{+}, 6-Rh^{3+}, 7-Pt^{2+}, 8-Pb^{2+}, 9-AsO_2^{-}, 10-AsO_4^{3-}, 11-Au^{3+}, 12-Pd^0 (1 μM), 13-Pd^0 (5 μM), 14-Pd^0 (10 μM), 15-Pd^0 (10 μM)+Ag^{+}+Pt^{2+}+Au^{3+}, 16- Pd^0 (10 μM)+Ru^{3+}+Rh^{3+}+Pb^{2+} and 17-Pd^0 (10 μM)+Cd^{2+}+Hg^{2+}+AsO_2^{-}+AsO_4^{3-}. Concentrations of the metal ions except Pd^0 were used as 100 μM. $Pd(PPh_3)_4$ was used as Pd^0 source.

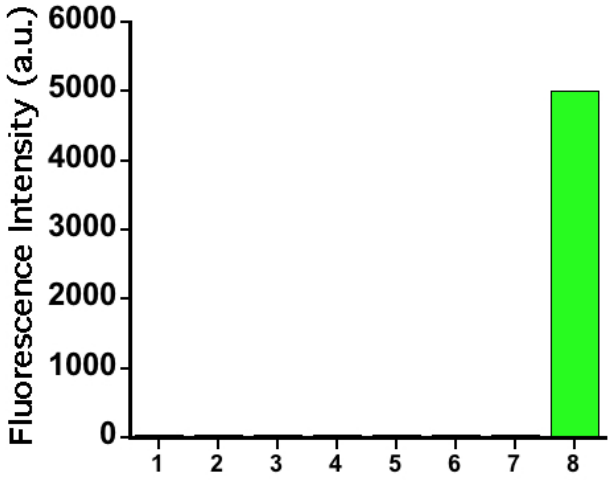


Fig. S8 Fluorescence responses (460 nm) of PCO-1 (10 μM) to palladium metal sources of different oxidation states: 1-none, 2-$PdCl_2$, 3-$Pd(OAc)_2$, 4-$Pd(CH_3CN)_2Cl_2$, 5-$Pd(PPh_3)_2Cl_2$, 6-Na_2PdCl_2, 7-$(NH_4)_2PdCl_6$ and 8-$Pd(PPh_3)_4$ incubated for 30 min in reaction buffer, of pH 8.0 at 37 °C (ex: 340 nm).

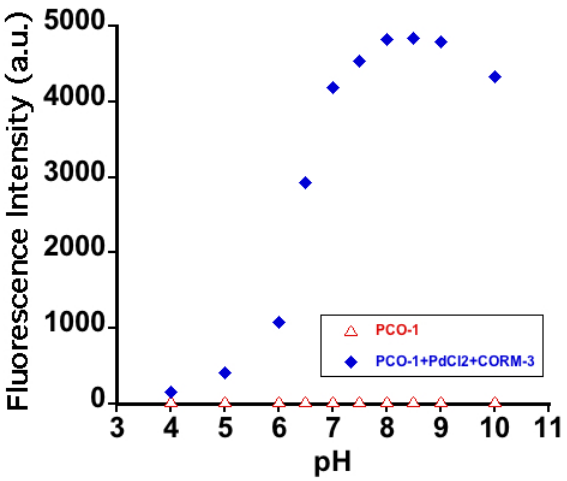


Fig. S9 Fluorescence responses (460 nm) of PCO-1 (10 μM) to CO (CORM-3, 50 μM) in presence of 10 μM $PdCl_2$ incubated for 30 min in reaction buffer of various pH at 37 °C (ex: 340 nm).

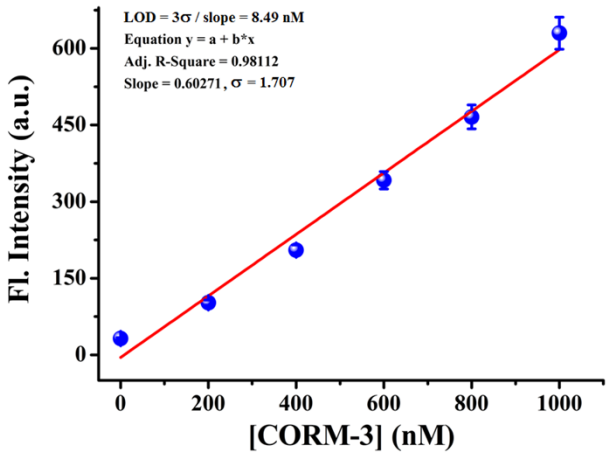


Fig. S10 Plot for the determination of limit of detection (LOD) for CO.

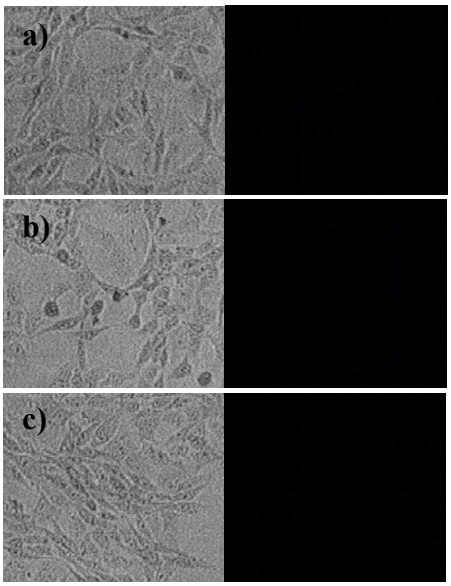


Fig. S11 Fluorescence microscopy images of A549 cells for control experiment using PCO-1 (10 μM) with the incubation of a) none, b) 10 μM $PdCl_2$ and c) 10 μM CORM-3 in the reaction buffer at 37°C (ex: ~340 nm). The first and second column represented the phase contrast and fluorescence images respectively.

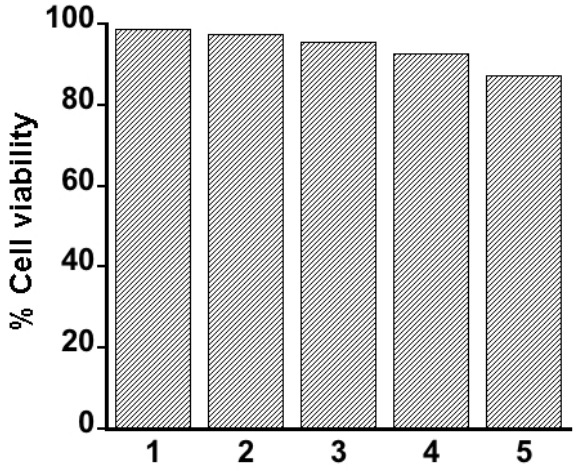
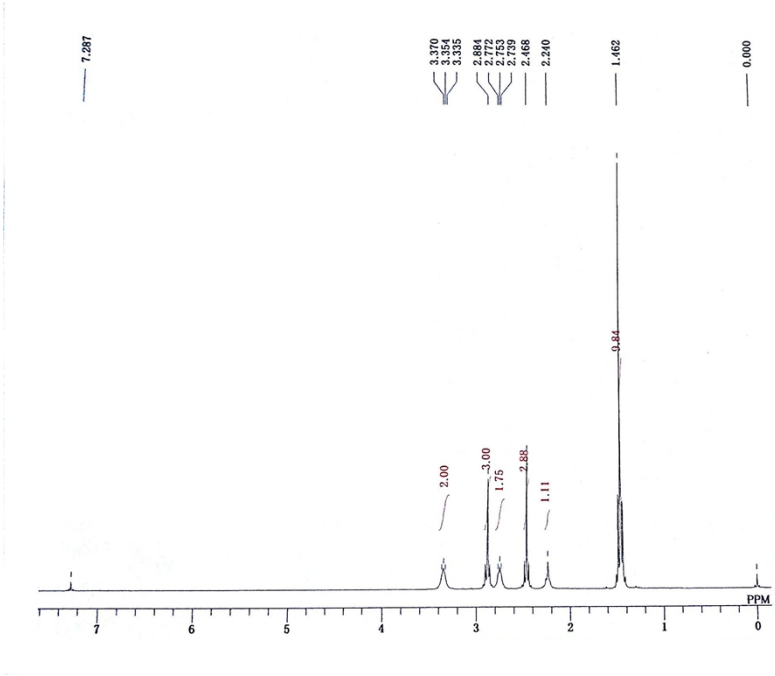


Fig. S12 Cytotoxic effect of PCO-1 (1: control, 2: 5 μM, 3: 10 μM, 4: 20 μM and 5: 50 μM) in A549 cells incubated for 4 h.

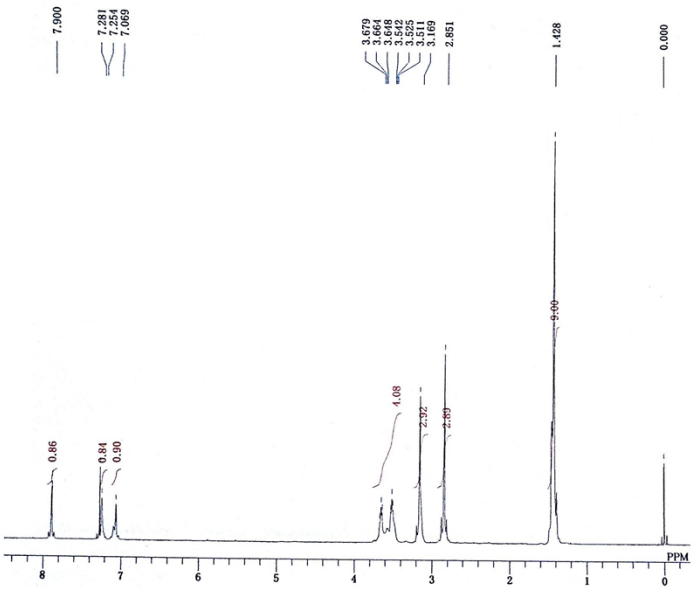
References:

1. E. Austin and M. Gouterman, *Bioinorg. Chem.*, 1978, **9**, 281.
2. I. B. Berlman, Handbook of Fluorescence Spectra of Aromatic Molecules, 1971, Academic Press, N.Y.
3. T. Mossman, *J. Immunol. Methods*, 1983, **65**, 55.

^{1}H and ^{13}C NMR of Compound 1 to PCO-1:



1H NMR of Compound 1



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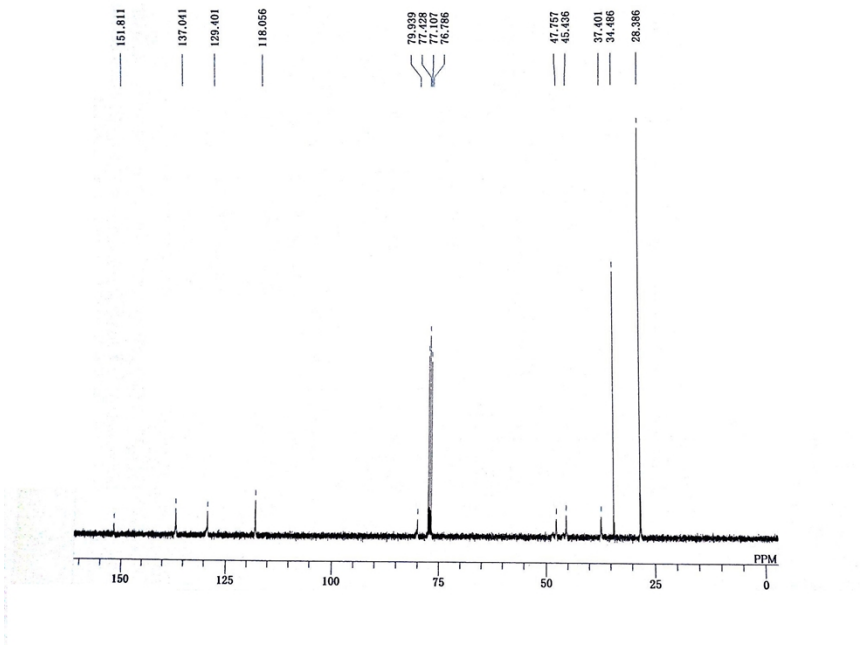
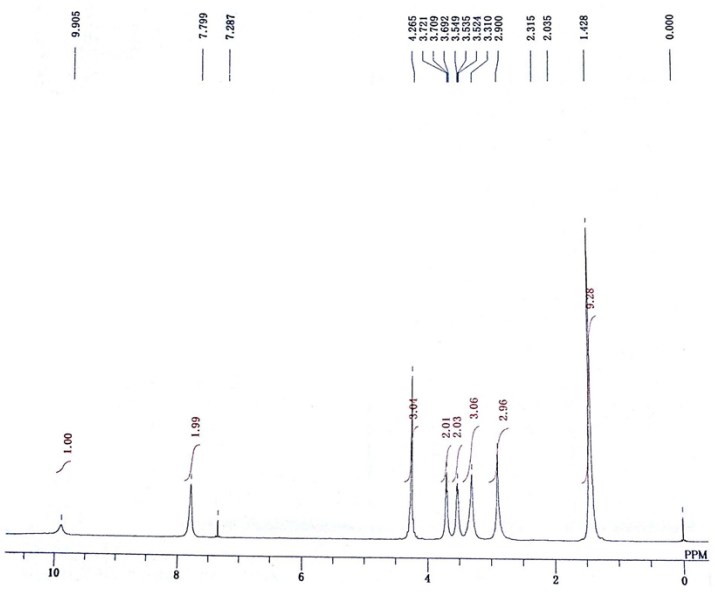
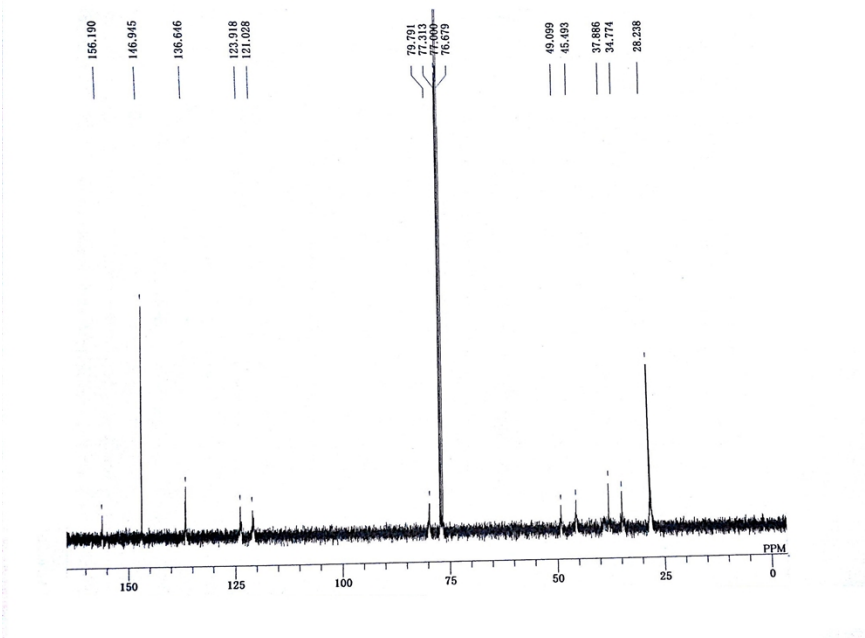


13C NMR of Compound 2



1H NMR of Compound 3



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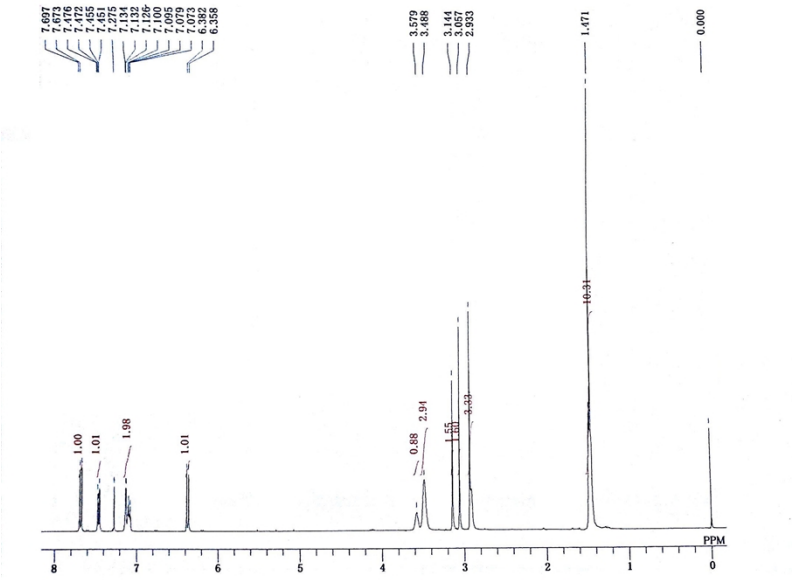
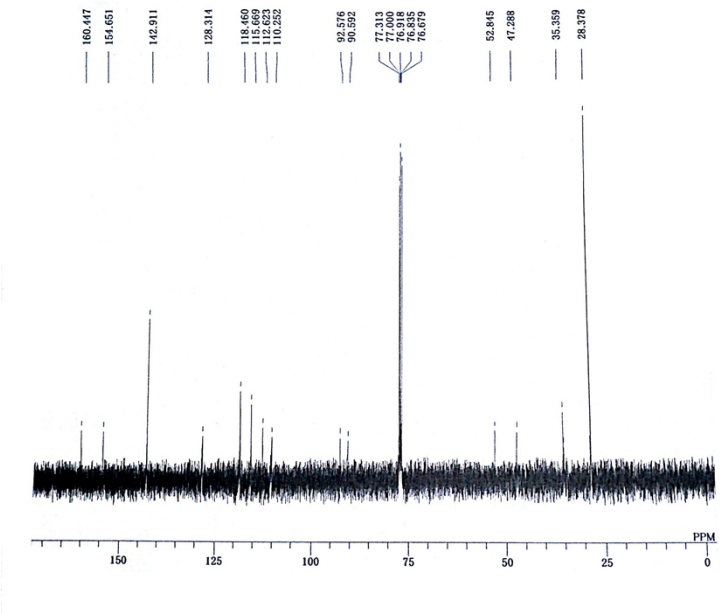


1H NMR of Compound 4



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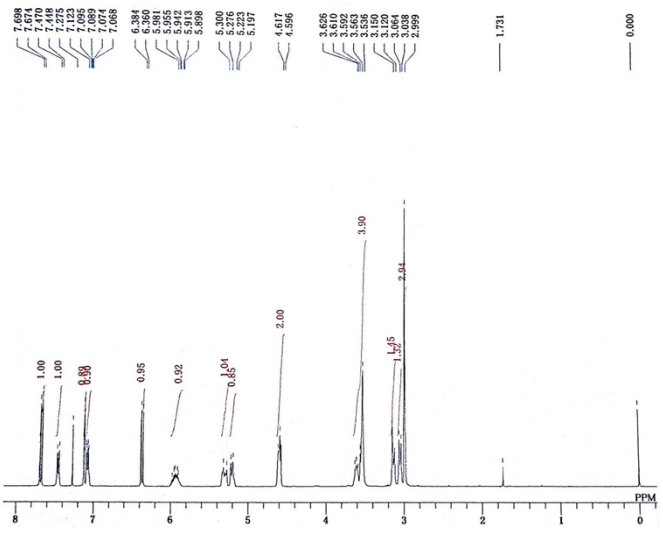
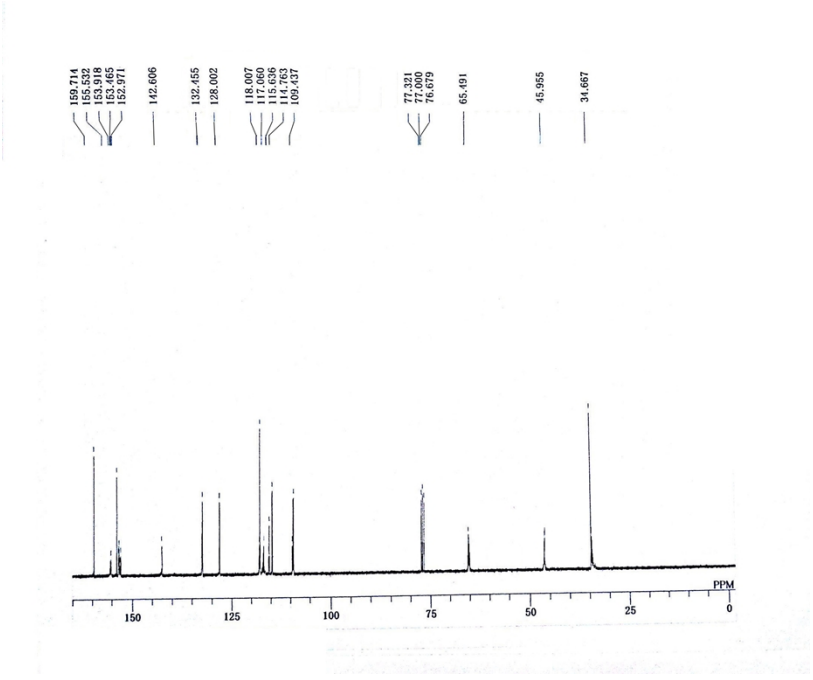


1H NMR of PCO-1



13C NMR of PCO-1