Ratiometric Determination of Nitroxyl Utilizing a Novel Fluorescence Resonance Energy Transfer-Based Fluorescent Probe Based on a Coumarin-Rhodol Derivative

Junhong Xu, Yu Bai, Qiujuan Ma,* Jingguo Sun, Meiju Tian, Linke Li, Nannan Zhu, and Shuzhen Liu

Cite This: ACS Omega 2022, 7, 5264−5273

ABSTRACT: Nitroxyl (HNO) is a member of the reactive nitrogen species, and how to detect it quickly and accurately is a challenging task. In this work, we designed and prepared a fluorescent ratiometric probe based on the fluorescence resonance energy transfer (FRET) mechanism, which can detect HNO with high selectivity. The coumarin derivative was used as an energy donor, the rhodol derivative was applied as an energy receptor, and 2-(diphenylphosphine)benzoate was utilized as the recognition group to detect nitroxyl. In the absence of HNO, the rhodol derivative exists in a non-fluorescent spironolactone state, and the FRET process is inhibited. Upon adding HNO, the closed spironolactone form is transformed into a conjugated xanthene structure and the FRET process occurs. This probe could specifically recognize nitroxyl, showing high sensitivity and selectivity. When the HNO concentration was changed from $3.0 \times 10^{-7}$ to $2.0 \times 10^{-5}$ mol·L$^{-1}$, $I_{543\text{nm}}/I_{470\text{nm}}$ exhibited a satisfactory linear correlation with the concentration of HNO. A detection limit of $7.0 \times 10^{-8}$ mol·L$^{-1}$ was obtained. In addition, almost no cell toxicity had been verified for the probe. The probe had been successfully applied to the ratiometric fluorescence imaging of HNO in HepG2 cells.

INTRODUCTION

Nitric oxide (NO), as a well-known signaling agent in many physiological processes, participates in physiological processes such as blood pressure control, neurotransmission, and immune response.\textsuperscript{1−4} Nitroxyl (HNO), as a single-electron reduction product or protonated product of NO,\textsuperscript{5−7} has also attracted the attention of researchers and become a research hotspot in modern biology. Nitroxyl can be directly produced by nitric oxide synthase under appropriate conditions,\textsuperscript{8,9} and play a very vital role in various physiological and pharmacological processes. For instance, HNO restrains the activity of aldehyde dehydrogenase through the interaction with protein thiol.\textsuperscript{10} Furthermore, HNO can be used as a vasodilator and positive inotropic drug to treat heart failure and induce vasodilation by up regulating the calcitonin gene-related peptide.\textsuperscript{11,12} Moreover, HNO is also used to treat alcoholism in clinic,\textsuperscript{13} relieve adverse reactions caused by ischemia−reperfusion injury,\textsuperscript{14} and functionalize as a potential anti-cancer drug.\textsuperscript{15} Thus, developing an effective assay for determining HNO is in demand in biological systems.

A variety of methods for detecting nitroxyl have been developed, such as mass spectrometry,\textsuperscript{16} high-performance liquid chromatography,\textsuperscript{17,18} colorimetric analysis,\textsuperscript{19,20} electrochemical method,\textsuperscript{21,22} electron paramagnetic resonance spectroscopy,\textsuperscript{23,24} fluorescence spectroscopy,\textsuperscript{25−28} and so on. Among these detection methods, the fluorescent probe technology processes many advantages including its superior sensitivity, non-invasive detection, real-time imaging, etc., and has become a powerful detection tool for HNO.\textsuperscript{29−31} So far, various sensing mechanisms have been widely applied in the
Scheme 1. Preparation Route of Probe 1

(a) Nitrobenzene, anhydrous AlCl₃, 84%; (b) m-hydroxyphenylpiperazine, CF₃COOH, 75%; (c) diethyl malonate, hexahydropyridine, 81%; (d) I. NaOH, II. HCl, 75%; (e) EDC, DMAP, 43%; (f) anhydrous CH₂Cl₂, EDC, DMAP, 47%.

Figure 1. Fluorescence spectra of probe 1 (10 μM) at different concentrations of AS (λex = 418 nm). Numbers 1−15 refer to salt concentrations of AS of 0, 0.3, 0.5, 0.7, 1.0, 15, 20, 30, 40, 50, and 60 μM, respectively. Illustration: scatter plot of I₄₅₄nm/I₄₇₀nm of probe 1 (10 μM) in the presence of different concentrations of AS.
construction of fluorescent probes, and these include the interaction of HNO with a metal complex or a metal porphyrin, 32−34 a thiol, 35,36 a phosphine, 23,26,37,38 or a nitrosocompound. 39 These investigations have extremely accelerated the advancement of HNO fluorescent probes. However, the currently reported fluorescent probes for detecting HNO are mostly designed based on the enhancement or attenuation of the fluorescence intensity of a single fluorophore, which are susceptible to uncertainties such as the probe concentration, external environment, and instrument sensitivity. 37 In terms of HNO detection accuracy in life, it only reaches the level of semi-quantitative analysis, so it is difficult to accurately measure the concentration of HNO in life. However, small differences in the HNO concentration in life may cause or reflect different physiological or pathological conditions. Thus, it is a particularly significant problem to construct a new type of fluorescent probe with excellent performance to meet the quantitative detection requirements of HNO.

For the sake of exploiting a fluorescent probe that overcame the above disadvantages, we constructed and prepared a fluorescent probe based on the FRET mechanism. The ratiometric fluorescent probe can avoid the defects caused by the single-wavelength fluorescent probe, and the ratiometric fluorescent probe with the FRET mechanism can realize the dual-wavelength quantitative detection of the analyte more accurately under single-wavelength laser excitation. 40 Nevertheless, only few fluorescent probes based on the FRET mechanism have been studied for the ratiometric detection of HNO. 40−42 Thus, ratiometric determination of nitroxylderiving FRET-based fluorescent probes is in demand for more investigating the function of HNO.

In our work, we constructed and prepared a ratiometric fluorescent probe 1 based on the FRET mechanism for highly selective determination of HNO (Scheme 1). A coumarin derivative with good optical properties was chosen as a resonance energy donor, a rhodol derivative was utilized as a resonance energy recipient, and 2-(diphenylphosphino)benzoate was applied as a recognition unit for sensing HNO. In the absence of HNO, the rhodol energy receptor existed in a resonance energy recipient induced by HNO. It is worth noting that the large emission shift (Δλ = 73 nm) resulted in two emission bands with good resolution for the probe, which would contribute to the dual-channel imaging of HNO in biological samples and detected less crosstalk. FRET efficiency is the vital factor of the FRET dye, which represents the energy transfer efficiency from the donor to the receptor. The fluorescence emission intensities of compound 5 (10 μM) and the reaction mixture of probe 1 (10 μM) with AS (50 μM) at 470 nm were 8222 and 366, respectively (Figure S1, Supporting Information). The fluorescence resonance energy transfer efficiency was found to be 96.0%, referring to energy transfer efficiency (ETF) = [(the fluorescence of donor − fluorescence of the donor in assette)/fluorescence of the donor] × 100%. 35

We further investigated the UV−vis absorption spectra of probe 1 (10 μM), the reaction mixture of fluorescent probe 1 (10 μM) with AS (50 μM), and compound 6 (30 μM) (Figure 2). As shown in Figure 2, probe 1 (10 μM) displayed a maximum absorption peak at 407 nm, which matched the absorption of the coumarin donor, 43 and compound 6 showed the absorption peaks at both 407 and 511 nm. Upon adding HNO, the absorption wavelength of the donor did not obviously change, but a new absorption peak at 511 nm arose, which corresponded to the conjugated xanthene form of the receptor. 40 Meanwhile, with HNO, the solution varied from colorless to orange, allowing colorimetric determination of HNO by naked eyes. We inferred that the probe 1 may react with the AS to generate compound 6.

**RESULTS AND DISCUSSION**

**Spectroscopic Analytical Performance of Probe 1 toward HNO.** Angeli’s salt (AS) was chosen as the source of HNO in our experiments. We investigated the fluorescence response of probe 1 (10 μM) to AS in a 0.01 M PBS buffer (CH3CN/H2O = 6:4, V/V, pH = 7.40) (Figure 1). From Figure 1, with the increase in the AS concentration, the fluorescence intensity at 470 nm decreased while that at 543 nm increased. The change occurred possibly because there was a FRET process between the coumarin energy donor and the rhodol energy recipient induced by HNO. It is worth noting that the large emission shift (Δλ = 73 nm) resulted in two emission bands with good resolution for the probe, which would contribute to the dual-channel imaging of HNO in biological samples and detected less crosstalk. FRET efficiency is the vital factor of the FRET dye, which represents the energy transfer efficiency from the donor to the receptor. The fluorescence emission intensities of compound 5 (10 μM) and the reaction mixture of probe 1 (10 μM) with AS (50 μM) at 470 nm were 8222 and 366, respectively (Figure S1, Supporting Information). The fluorescence resonance energy transfer efficiency was found to be 96.0%, referring to energy transfer efficiency (ETF) = [(the fluorescence of donor − fluorescence of the donor in assette)/fluorescence of the donor] × 100%. 35

![Figure 2. Absorption spectra of fluorescent probe 1 (10 μM), compound 6 (30 μM), and the reaction mixture of fluorescent probe 1 (10 μM) with AS (50 μM), and compound 6 (30 μM) (Figure 2).](https://doi.org/10.1021/acsomega.1c06403)
is $I_{543\text{nm}}/I_{470\text{nm}} = 0.0457 + 0.1081 \times 10^6 \times C \ (r = 0.9976)$, where $C$ represents the concentration of AS and $r$ is the linear correlation coefficient. The detection limit was calculated by $3S_B/m$ (where $S_B$ is the standard deviation of 10 blank solution measurements and $m$ is the slope of the linear regression equation). The detection limit was found to be $7.0 \times 10^{-8}$ mol·L$^{-1}$, which is much lower than the previously developed ratiometric fluorescent probes for HNO.37,40,42,44 The results demonstrated that probe 1 could be utilized for highly sensitive quantitative determination of HNO by a ratiometric manner.

According to the previously reported references,25,26,37,38 we inferred that the reaction mechanism of probe 1 to HNO may be attributed to the reaction of probe 1 and AS to produce compound 6 (Scheme 2). As we can see from Scheme 2, without HNO, the rhodol receptor existed in the non-fluorescent lactone state and the FRET process was suppressed. After adding HNO, HNO interacted with the 2-(diphenylphosphino)benzoate unit of probe 1 to produce the corresponding aza-ylide, which could nucleophilically attack the carbonyl of the ester in an intramolecular manner to yield hydroxyl groups. Therefore, in the presence of HNO, the closed spirolactone form was converted to a conjugated fluorescent xanthene structure to restore the FRET process. For the sake of further confirming the detection mechanism of probe 1 for HNO, probe 1, the reaction mixture of probe 1 with AS, and compound 6 were further analyzed by HPLC (Figure 4). From Figure 4, the synthesized probe 1 peaked at 7.38 min, the reaction mixture of probe 1 and AS displayed a chromatogram peak at 2.97 min, and compound 6 also peaked at 2.97 min. It can be seen that the retention time of the reaction mixture of probe 1 and AS is consistent with that of compound 6. Compound 6 could be provided by the reaction of probe 1 with AS, which had been verified by the above HPLC experiments. Meanwhile, the reaction mixture of probe 1 and AS displayed the same maximum absorption wavelength as compound 6 in the UV–vis absorption spectra.

Additionally, the reaction mixture of probe 1 and AS was further analyzed by mass spectrometry (Figure S2). From Figure S2, three peaks at $m/z$ 322.0987, 644.2400, and 948.3048 were seen, which corresponded to the $[\text{phosphonylbenzamide} + \text{H}]^+$, $[6 + \text{H}]^+$, and $[\text{the corresponding oxide} + \text{H}]^+$, respectively. Thus, the above results verified that the proposed detection mechanism of probe 1 for AS was right.

**Time-Dependent Responses.** The time response of probe 1 to AS was studied by the trend of $I_{543\text{nm}}/I_{470\text{nm}}$ changes with time before and after adding 50 μM AS (Figure 5). As shown in Figure 5, when AS was not added, there was no change in the $I_{543\text{nm}}/I_{470\text{nm}}$ value. When AS was added, the $I_{543\text{nm}}/I_{470\text{nm}}$ value enhanced with time and gained a plateau at 25 min. In addition, upon adding HNO, the fluorescence color change from blue to yellow could be observed under ultraviolet light. During this test, $I_{543\text{nm}}/I_{470\text{nm}}$ was recorded after adding HNO for 30 min.
To obtain information of the pH effects, we investigated variations in the $I_{543\text{nm}}/I_{470\text{nm}}$ values of synthesized probe 1 (10 μM) in the absence and presence of HNO (20 μM) at different pH values (Figure 6). As illustrated in Figure 6, probe 1 has a good response to AS between pH 5.00 and 10.00. Consequently, experimental results demonstrated that probe 1 could function in a wide pH scope and could determine HNO in biological samples.

**Selectivity.** The selectivity of the fluorescent probe determines its usability in the actual sample. Therefore, we conducted a selective inspection of probe 1 by studying the changes of $I_{543\text{nm}}/I_{470\text{nm}}$ of probe 1 for AS and other related substances at pH 7.40 (Figure 7A). As we can see from Figure 7A, with the addition of 50 μM AS, $I_{543\text{nm}}/I_{470\text{nm}}$ of the probe increased significantly. By contrast, after treatments with other substances, $I_{543\text{nm}}/I_{470\text{nm}}$ did almost not change. Furthermore, the variations of $I_{543\text{nm}}/I_{470\text{nm}}$ of probe 1 were also examined when other species coexisted with AS (Figure 7B). As displayed in Figure 7B, compared with only AS, no obvious variation of $I_{543\text{nm}}/I_{470\text{nm}}$ was seen in the coexistence of other substances and AS. The above results indicated that the probe had a high selectivity for HNO and owned the ability to determine HNO in complicated biological samples.

**Cytotoxicity Assays and Confocal Imaging in Living Cells.** To investigate the cytotoxicity of probe 1, the MTT assay was applied to evaluate the cytotoxicity of probe 1 and compound 6 on HepG2 cells (Figure 8). As seen from Figure 8, probe 1 and compound 6 at different concentrations were added to the culture medium containing HepG2 cells for 24 h
and the cell survival rate reached over 85%, indicating that probe 1 and compound 6 had almost no toxicity to living cells.

Then, on this basis, double-channel ratio-type fluorescence imaging was performed on probe 1 to detect HNO in living cells (Figure 9). From Figure 9, when the HepG2 cells were incubated with 10 μM probe 1 for 30 min and imaged, the blue channel exhibited blue fluorescence (Figure 9b) and the red channel showed weak fluorescence (Figure 9c). In control experiments, the HepG2 cells were incubated with 10 μM probe 1 for 30 min and then treated with 50 μM AS for 45 min. The control experiments showed that the blue channel displayed weaker blue fluorescence (Figure 9f), and the red channel emitted stronger red fluorescence (Figure 9g). These results demonstrated that the ratiometric probe 1 could be utilized to sense HNO in living cells by the dual-channel imaging mode.

**CONCLUSIONS**

In a word, a FRET-based ratiometric probe for determining HNO based on a coumarin-rhodol derivative has been constructed and prepared. The 2-(diphenylphosphino)-benzoate group was chosen as a sensing unit for HNO. In the absence of HNO, the rhodol receptor existed in the non-fluorescent lactone state and the FRET process was suppressed. Upon adding HNO, the closed spirolactone form was transformed to a conjugated fluorescent xanthene form to result in the occurrence of FRET, which induced a fluorescence intensity decrease at 470 nm and increase at 543 nm. The probe illustrated high sensitivity and selectivity for HNO. Furthermore, the probe displayed almost no toxicity to living cells and had been effectively used to sense HNO in living cells by the ratiometric dual-channel imaging mode.

**EXPERIMENTAL SECTION**

Materials and Instruments. 4-(Diethylamino)-salicylaldehyde, m-diphenol, diethyl malonate, phthalic anhydride, 2-(diphenylphosphino)benzoic acid, trifluoroacetic acid, and 4-diaminopyridine (DMAP) were supplied by Heowns Biochemical Technology Company. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and m-hydroxyphenylpiperazine were obtained from Energy Chemical (Shanghai, China). n-Hexane and anhydrous...
aluminum chloride were bought from Tianjin Sailboat Chemical Reagent Technology Company. Nitrobenzene was bought from the Tianjin Damao Chemical Reagent Factory. Hexahydropyridine was purchased from Shanghai Pharmaceutical Reagent Company of the China Pharmaceutical Group. Triethylamine was provided by the Tianjin Fuchen Chemical Reagent Factory. The chromatographically pure chemical reagents were analytically pure reagents, purchased from commercial suppliers and used directly in the experiment without further purification. Silica gel 60 F254 was used in thin layer chromatography, and 20–300 mesh silica gel was applied in column chromatography, both of which were obtained from Tianjin Damao Chemical Reagent Factory. Nitrobenzene was bought from Tianjin Sailboat Chemical Reagent Technology Company. A Waters LC 2695-2998 HPLC/UV instrument was utilized to measure the UV-visible spectrophotometry and UV-visible spectrophotometry were measured in 0.01 mol/L PBS buffer (CH3CN/water = 6:4, V/V, pH = 7.40). Except for the fluorescence data obtained by time scanning, all other fluorescence and absorption data were measured at 30 min after addition of HNO at room temperature.

Syntheses. The prepared process for FRET-based ratiometric fluorescent probe 1 for highly selective determination of HNO is displayed in Scheme 1. Compounds 2–6 were synthesized referring to the previous reports.43,45 The prepared detail and the corresponding characterization data were listed below. Probe 1 was constructed based on the coumarin-rhodol FRET system. The UV–vis absorption spectrum of the rhodol energy receptor (compound 3) could be successfully overspread by the fluorescence emission spectra of the coumarin energy donor (compound 5),45 which laid the foundation for fluorescence resonance energy transfer of the coumarin donor to the rhodol acceptor.

Synthesis of Compound 1. Under the protection of nitrogen, 2-(diphenylphosphino)benzoic acid (0.31 g, 1 mmol) were supplied. The reaction concoction was stirred at room temperature for 30 min. Compound 6 (0.77 g, 1.2 mmol) was supplied, and the reaction concoction was stirred for 12 h. A crude material was obtained by the evaporation of the reaction solution under reduced pressure. A yellow solid compound 1 (0.44 g, 47%) was yielded through column chromatography, purifying the above crude material. Dichloromethane/methanol (25:1, V/V) was used as the eluent. 1H NMR (500 MHz, CDCl3), δ(ppm): 8.23–8.22 (1H, m), 7.99 (2H, d, J = 7.5 Hz), 7.88 (1H, s), 7.65–7.57 (2H, m), 7.47–7.42 (2H, m), 7.32–7.26 (10H, m), 7.13 (1H, d, J = 7.5 Hz), 6.98–6.96 (1H, m), 6.91 (1H, d, J = 1.75 Hz), 6.71 (2H, d, J = 8.8 Hz), 6.66–6.57 (4H, m), 6.47 (1H, s), 3.90 (2H, s), 3.57 (2H, s), 3.48 (4H, q, J = 7.1 Hz), 3.33 (4H, d, J = 15.2 Hz), 1.24–1.19 (6H, m) (Figure S3, Supporting Information). 13C NMR (125 MHz, CDCl3), δ(ppm): 171.12, 169.39, 165.06, 164.48, 159.14, 157.33, 153.04, 152.38, 151.86, 151.73, 151.68, 145.50, 141.64, 141.42, 137.46, 137.37, 135.02, 134.44, 134.06,
CaCl2, FeCl3, Na2S, and ZnCl2 in twice-distilled water.

Concentrations of probe matrix was then removed, washed three times with Dulbecco anhydrous DMSO. Singlet oxygen (1O2) was supplied by reacting 0.2 mM Fe2+ with 200 μM TBHP. Peroxynitrite (ONOO−) solution was prepared referring to the described method, and its concentration was evaluated by recording the absorbance of the experimental group and the control group, respectively.

Preparation of Samples and Test Solutions. The stock solutions of probe 1 and compound 6 were supplied by dissolving probe 1 and compound 6 in dimethylsulfoxide (DMSO) solution, respectively. The solutions of different test analytes were provided from cysteine (Cys), glutathione (GSH), homocysteine (Hcy), AlCl3, NaCl, KCl, MgCl2, CaCl2, FeCl3, Na2S, and ZnCl2 in twice-distilled water. Different oxidants were obtained according to the formerly reported references. HNO was obtained by dissolving Angeli’s salt (AS) in 0.01 M NaOH solution. Superoxide (O2−) was yielded by adding potassium superoxide (KO2) in anhydrous DMSO. Singlet oxygen (1O2) was supplied by reacting 1 mM OCl− with 200 μM H2O2. The hydroxyl radical (·OH) and tert-butoxy radical (·OBu) were prepared by reacting 0.2 mM Fe3+ with 200 μM H2O2 or 200 μM TBHP, respectively. The 10, 30, and 70% aqueous solutions were the sources of hypochlorite (ClO−), hydrogen peroxide (H2O2), and tert-butyl hydroperoxide (TBHP), respectively. Peroxynitrite (ONOO−) solution was prepared by the described method and its concentration was evaluated by utilizing an extinction coefficient of 1670 M−1·cm−1 at 302 nm in 0.1 M NaOH.

Cytotoxicity Assay. Cytotoxicity was studied by the MTT assay. First, HepG2 (hepatoma cells) cells were cultured using a CO2 incubator at 37 °C with a culture solution containing high concentrations of glucose, 10% fetal bovine serum, and compound 6. 104 cells per well were seeded on a 96-well plate, and the total volume of each well was controlled at 100 μL for 24 h. The matrix was then removed, washed three times with Dulbecco’s phosphate buffered saline (DPBS), and cultured with different concentrations of probe 1 and compound 6 for 24 h. Then, the medium was moved from the 96-well plate. MTT solution was added to each well, and the cells were hatched for another 4 h to guarantee formazan produce. Finally, the supernatant was removed, and the cells were swayed for 10 min after adding 150 μL of dimethylsulfoxide (DMSO). A microplate reader was utilized to measure the absorbance at 490 nm, and the cell survival rate of HepG2 was calculated referring to A/A0 × 100% (A and A0 represented the absorbance of the experimental group and the control group, respectively).

Confocal Imaging in Living Cells. To detect HNO in living cells, fluorescence imaging experiments of living cells were carried out. First, HepG2 cells were seeded in a laser confocal plate with a 35 mm diameter and cultured in an incubator for 12 h. After adding 10 μM probe 1, the living cells were incubated for 30 min. The medium was aspirated. Then, the HepG2 cells were imaged after being washed with DPBS three times. In the control experiment, after adding 10 μM probe 1, the living cells were cultured for 30 min. The culture solution was aspirated, and the cells were washed with DPBS three times. Next, the cells were incubated for another 45 min with 0.05 mM AS, cleaned three times with DPBS, and imaged. The confocal fluorescence image was observed with a 60X objective Olympus FV1200-MPE multiphoton confocal microscope.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06403.

Fluorescence emission spectra of compound 5 (10 μM) and the mixture of probe 1 (10 μM) and AS (50 μM) in 0.01 M PBS buffer (CH3CN/water = 6:4, V/V, pH = 7.40); ESI-HRMS spectrum of compound 1 (10 μM) in 0.01 M PBS buffer (CH3CN/water = 6:4, V/V, pH = 7.40) recorded at 30 min after the addition of AS (50 μM); and 1H NMR, 13C NMR, and ESI-MS spectra of compound 1 (PDF)

AUTHOR INFORMATION
Corresponding Author
Qijuan Ma — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China; orcid.org/0000-0001-9785-2733; Phone: +86-371-65676656; Email: maqiujuan104@126.com; Fax: +86-371-65680028

Authors
Junhong Xu — Department of Dynamical Engineering, North China University of Water Resources and Electric Power, Zhengzhou 450011, PR China
Yu Bai — School of Pharmacy and Chemical Engineering, Zhengzhou University of Industrial Technology, Zhengzhou 450011, PR China; School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China
Jingguo Sun — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China
Meiju Tian — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China
Linke Li — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China
Nannan Zhu — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China
Shuzhen Liu — School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, PR China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c06403

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 21807027), the Zhongjing Scholars Research Funding of Henan University of Chinese Medicine, and the Graduate Innovation Fund of the Henan College of Chinese Medicine (2021KYCX051).

REFERENCES

1. Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987, 327, 524−526.
2. Cannon, R. O., III Role of nitric oxide in cardiovascular disease: focus on the endothelium. Clin. Chem. 1998, 44, 1809−1819.
(3) Brod, D. S.; Hwang, P. M.; Snyder, S. H. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 1990, 347, 768–770.

(4) Kerwin, J. F., Jr.; Lancaster, J. R.; Feldman, P. L. Nitric oxide: a new paradigm for second messengers. J. Med. Chem. 1995, 38, 4343–4362.

(5) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(6) Scherlis, D.; Salvarezza, R. C.; Mart, M. A.; Doctorovich, F. A surface azanone (HNO) at low nanomolar level. ACS Omega 2020, 7, 5264–5273.

(7) Kerwin, J. F., Jr.; Lancaster, J. R.; Feldman, P. L. Nitric oxide: a new paradigm for second messengers. J. Med. Chem. 1995, 38, 4343–4362.

(8) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(9) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(10) Kerwin, J. F., Jr.; Lancaster, J. R.; Feldman, P. L. Nitric oxide: a new paradigm for second messengers. J. Med. Chem. 1995, 38, 4343–4362.

(11) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(12) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(13) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(14) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(15) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(16) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(17) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(18) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(19) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(20) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(21) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(22) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(23) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(24) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(25) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(26) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.

(27) Doctorovich, F.; Bikiel, D.; Pellegrino, J.; Suárez, S. A.; Larsen, A.; Martí, M. A. Nitroxyl (azanone) trapping by metalloporphyrins. Coord. Chem. Rev. 2011, 255, 2764–2784.
and tissues using a nitroxylic-responsive two-photon fluorescence probe. Anal. Chem. 2017, 89, 4587−4594.

(42) Zhang, H.; Liu, R.; Tan, Y.; Xie, W.; Cheung, H.-Y.; Sun, H. A FRET-based ratiometric fluorescent probe for nitroxylic detection in living cells. ACS Appl. Mater. Interfaces 2015, 7, 5438−5443.

(43) Bai, Y.; Wu, M. X.; Ma, Q. J.; Wang, C. Y.; Sun, J. G.; Tian, M. J.; Li, J. S. A FRET-based ratiometric fluorescent probe for highly selective detection of cysteine based on a coumarin-rhodol derivative. New J. Chem. 2019, 43, 14763−14771.

(44) Liu, C.; Wu, H.; Wang, Z.; Shao, C.; Zhu, B.; Zhang, X. A fast-response, highly sensitive and selective fluorescent probe for the ratiometric imaging of nitroxylic in living cells. Chem. Commun. 2014, 50, 6013−6016.

(45) Sun, J.; Bai, Y.; Ma, Q.; Zhang, H.; Wu, M.; Wang, C.; Tian, M. A FRET-based ratiometric fluorescent probe for highly selective detection of hydrogen polysulfides based on a coumarin-rhodol derivative. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 2020, 241, 118650.