

Electronic Supplementary Information for

Development of a urea-bond cleavage reaction induced by nitric oxide for its fluorescence imaging

Yuqing Zhang^{1,†}, Shushu Wang^{1,†}, Lina Zhang¹, and Tao Peng^{1,*}

¹ State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China

† These authors contributed equally.

* For correspondence: Tao Peng, tpeng@pku.edu.cn

Table of Contents

Supplementary figures	3
Experimental procedures	15
Photophysical characterization of probes	15
LC-MS analysis on reactions of fluorescent probes with NO	15
Cell culture	16
Confocal fluorescence imaging of NO in live cells	16
MTT assay	17
Fluorescence imaging of NO in living mice	18
Chemical synthesis	19
Synthetic materials and methods	19
Synthesis of probes WNO1–6	20
Synthesis of probes WNO7–16	29
Synthesis of probes WNO17–25	47
Synthesis of probes WNO26	68
Synthesis of probes WNO27	72
Synthesis of probes WNO28	75
References	77
NMR spectra	78

Supplementary figures

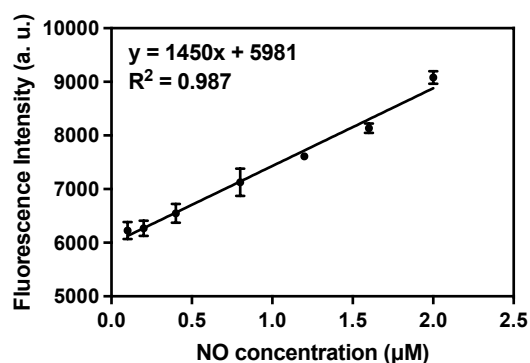


Figure S1. Fluorescence response of WNO26 (10 μM) to low concentrations of NO (0.1–2 μM) in PBS (20 mM, pH 7.4) at 37 °C for 1 h. The probe was excited at 440 nm and fluorescence intensity at 554 nm was recorded. Data are presented as mean ± standard deviation ($n = 3$).

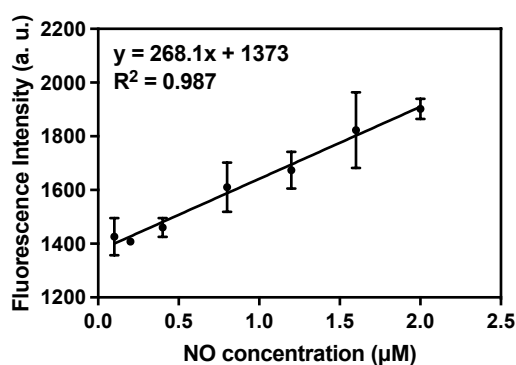


Figure S2. Fluorescence response of WNO27 (10 μM) to low concentrations of NO (0.1–2 μM) in PBS (20 mM, pH 7.4) at 37 °C for 1 h. The probe was excited at 492 nm and fluorescence intensity at 559 nm was recorded. Data are presented as mean ± standard deviation ($n = 3$).

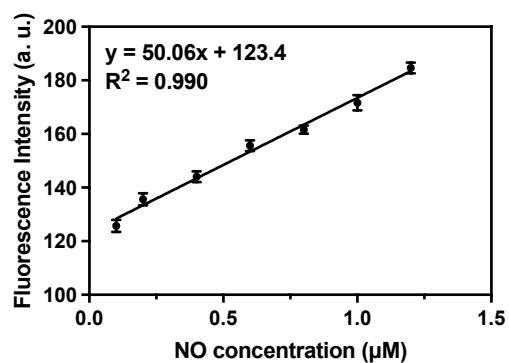


Figure S3. Fluorescence response of WNO28 (10 μM) to low concentrations of NO (0.1–1.2 μM) in PBS (20 mM, pH 7.4) at 37 $^{\circ}\text{C}$ for 1 h. The probe was excited at 674 nm and fluorescence intensity at 700 nm was recorded. Data are presented as mean \pm standard deviation ($n = 3$).

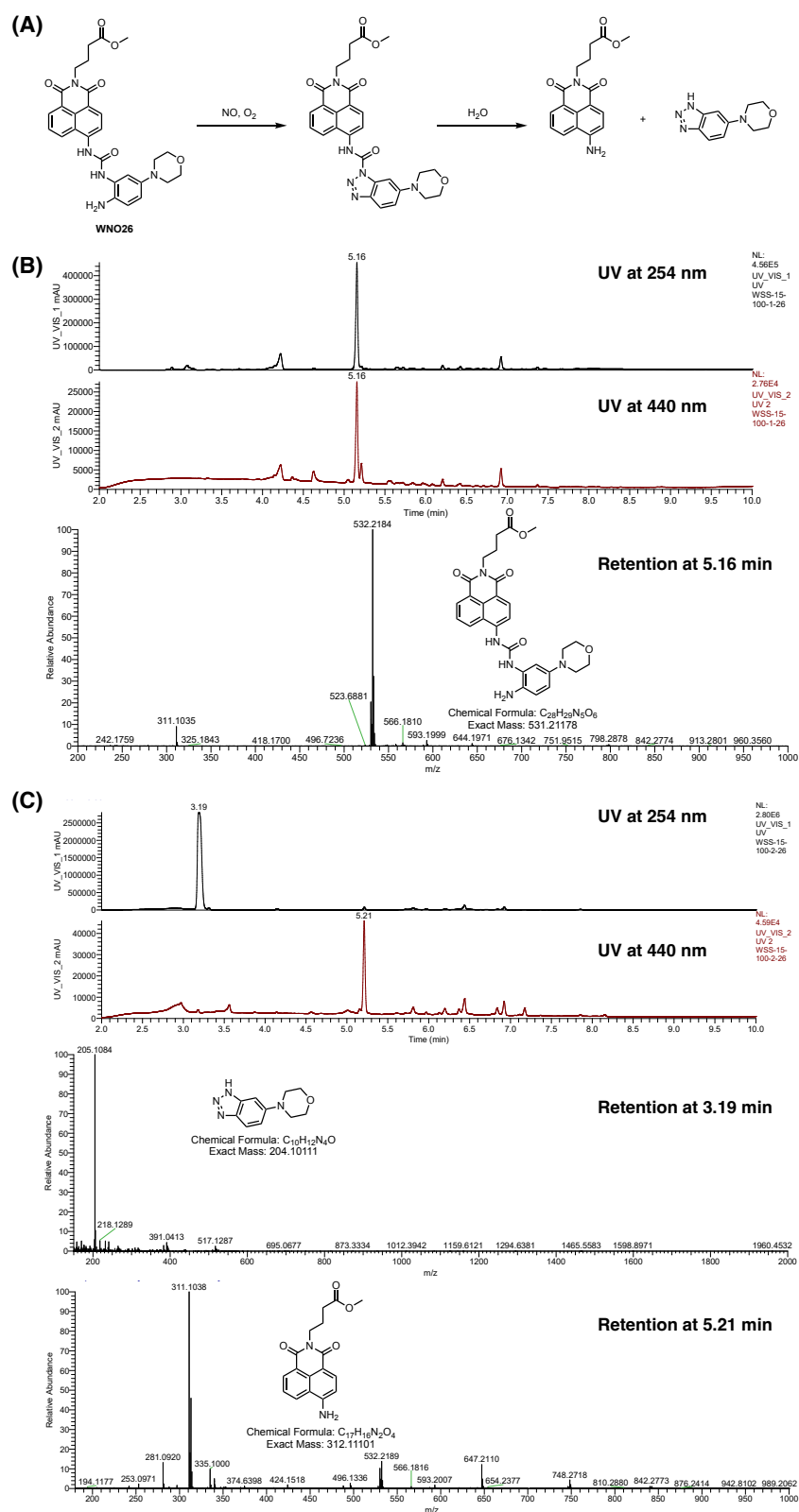


Figure S4. LC-MS analysis of the reaction between WNO26 with NO. (A) The reaction between WNO26 and NO. (B) LC and mass spectra of WNO26 before reaction with NO. (C) LC and mass spectra of the reaction mixture between WNO26 and NO, confirming the production of the fluorophore and benzotriazole byproduct.

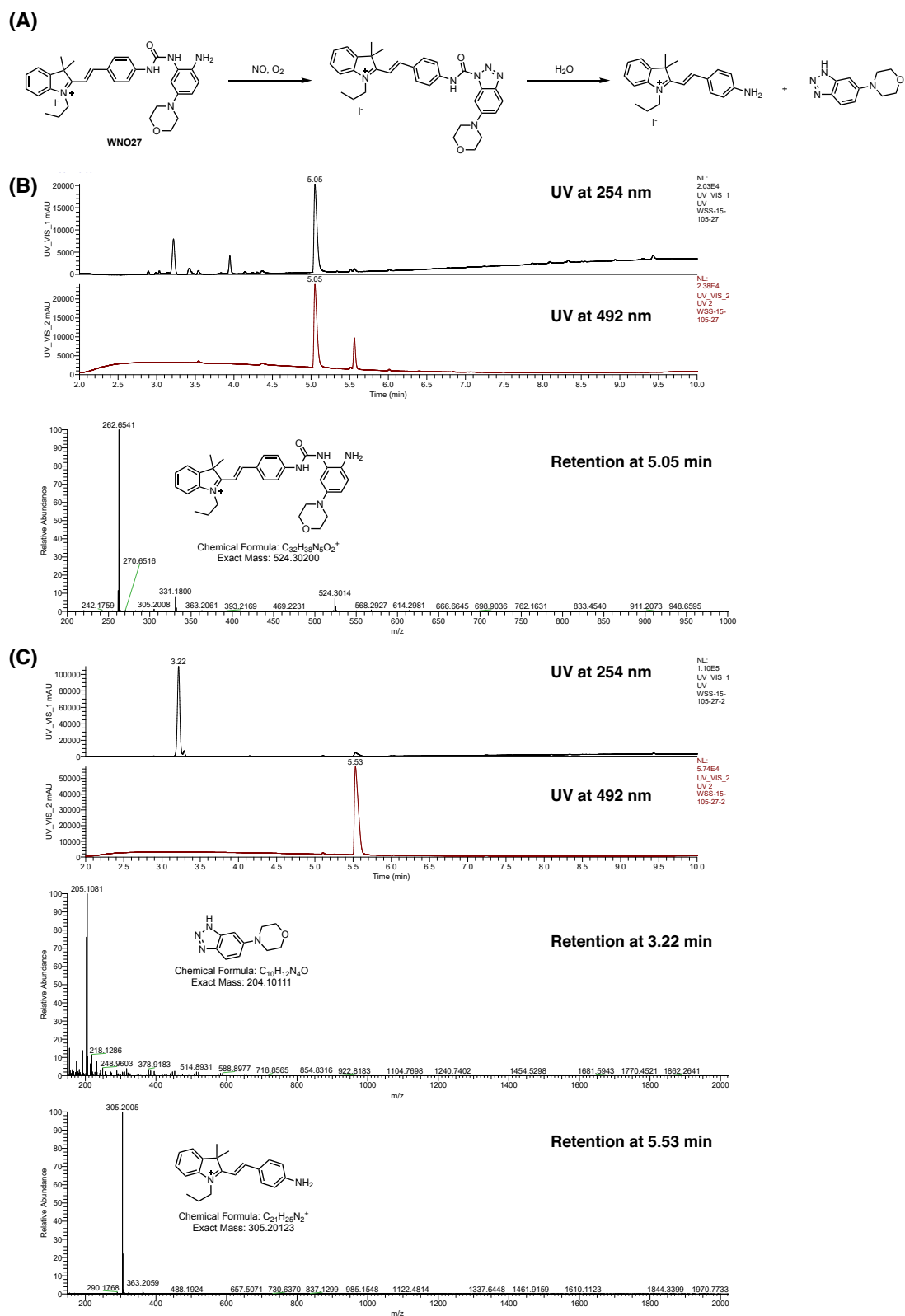


Figure S5. LC-MS analysis of the reaction between WNO27 with NO. (A) The reaction between WNO27 and NO. (B) LC and mass spectra of WNO27 before reaction with NO. (C) LC and mass spectra of the reaction mixture between WNO27 and NO, confirming the production of the fluorophore and benzotriazole byproduct.

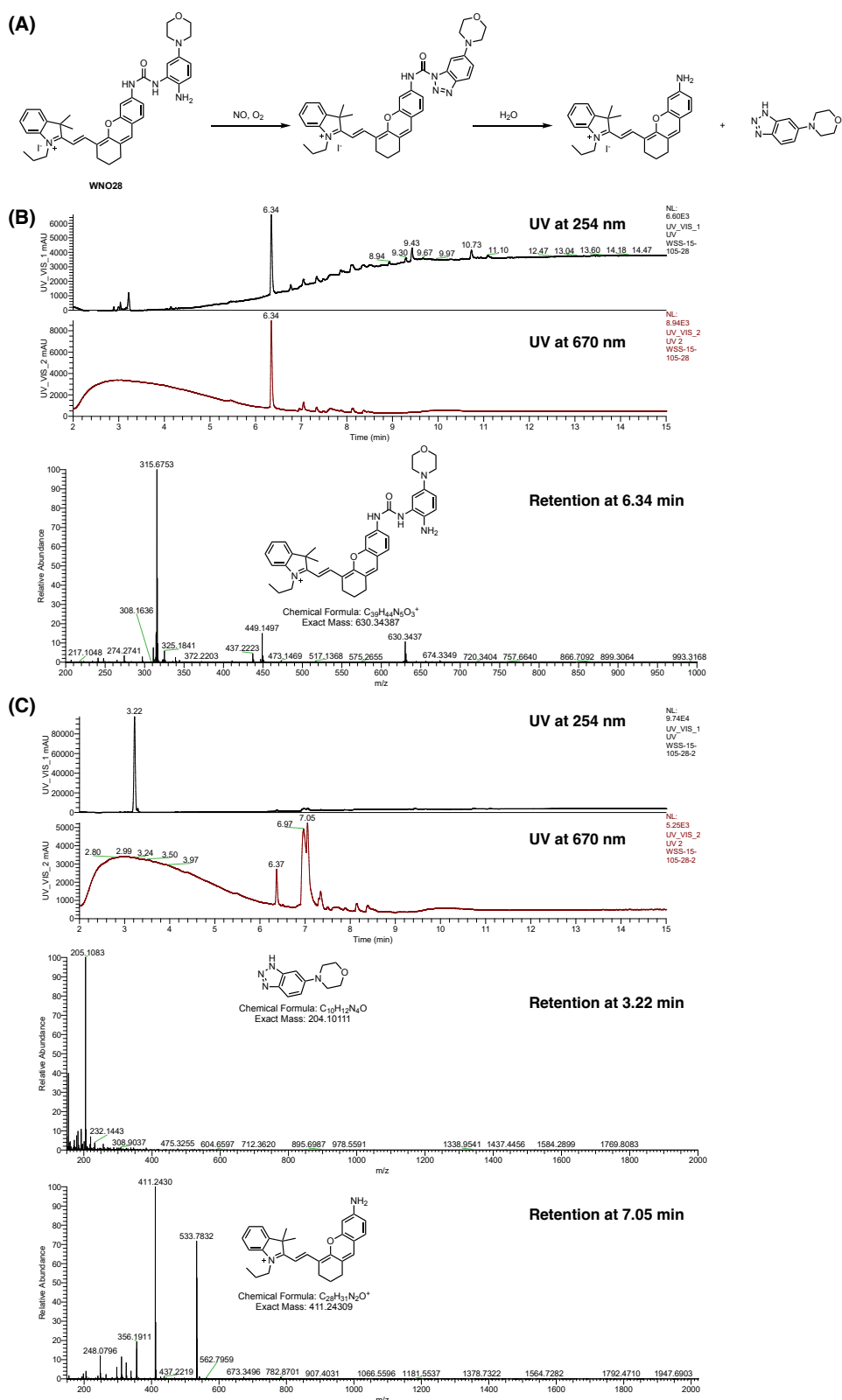


Figure S6. LC-MS analysis of the reaction between WNO28 with NO. (A) The reaction between WNO28 and NO. (B) LC and mass spectra of WNO28 before reaction with NO. (C) LC and mass spectra of the reaction mixture between WNO28 and NO, confirming the production of the fluorophore and benzotriazole byproduct.

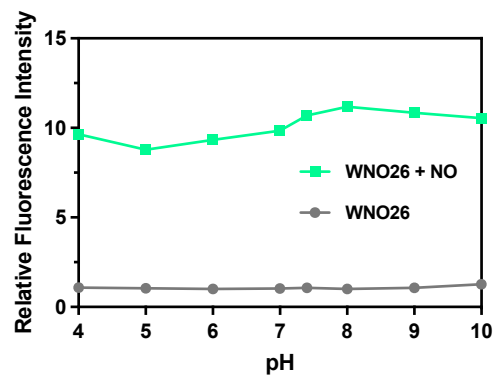


Figure S7. Fluorescence response of WNO26 (10 μ M) to NO in PBS (20 mM, pH 7.4) at different pH values at 37 $^{\circ}$ C for 1 h. The probe was excited at 440 nm and fluorescence intensity at 554 nm was recorded.

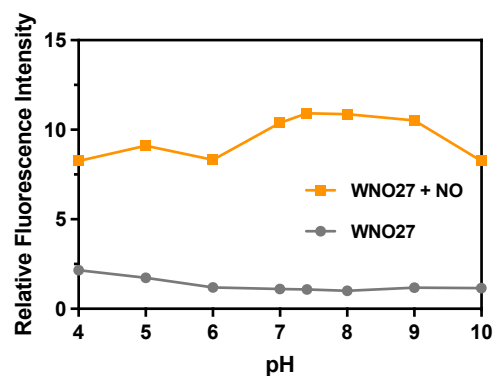


Figure S8. Fluorescence response of WNO27 (10 μ M) to NO in PBS (20 mM, pH 7.4) at different pH values at 37 $^{\circ}$ C for 1 h. The probe was excited at 492 nm and fluorescence intensity at 559 nm was recorded.

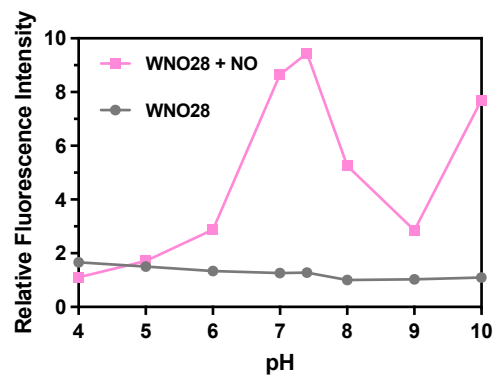


Figure S9. Fluorescence response of WNO28 (10 μ M) to NO in PBS (20 mM, pH 7.4) at different pH values at 37 $^{\circ}$ C for 1 h. The probe was excited at 674 nm and fluorescence intensity at 700 nm was recorded.

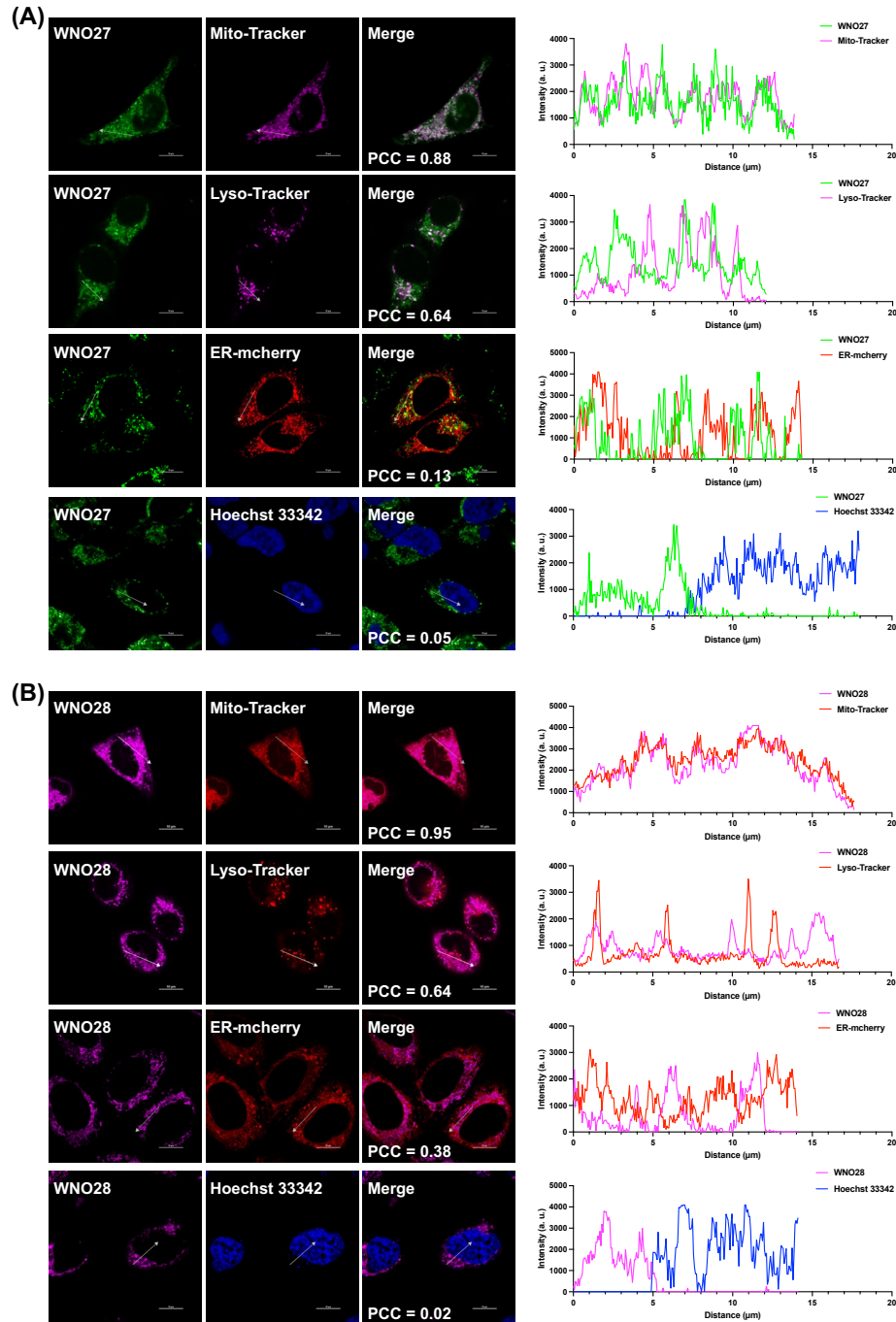


Figure S10. Fluorescence imaging of the colocalization of WNO27 and WNO28 with organelle markers. HeLa cells were incubated with (A) WNO27 (5 μ M) or (B) WNO28 (2 μ M) for 30 min and treated with DEA NONOate (100 μ M) for 60 min, followed by staining with Mito-Tracker, Lyso-Tracker, or Hoechst 33342, respectively. For staining ER, HeLa cells expressing ER-targeting mCherry were used. Scale bars represent 10 μ m. Pearson correlation coefficients (PCC) are shown in the images. Fluorescence intensity profiles of WNO27 and WNO28 with the corresponding organelle markers are shown on the right.

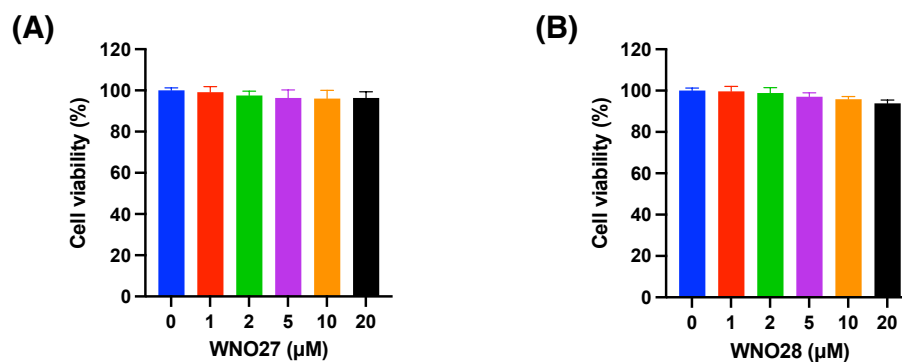


Figure S11. MTT analysis of the cytotoxicity of WNO27 and WNO28. HeLa cells were incubated with (A) WNO27 or (B) WNO28 at varying concentrations for 24 h and subjected to the MTT assay. Cell viabilities are shown as mean \pm standard deviation ($n = 7$).

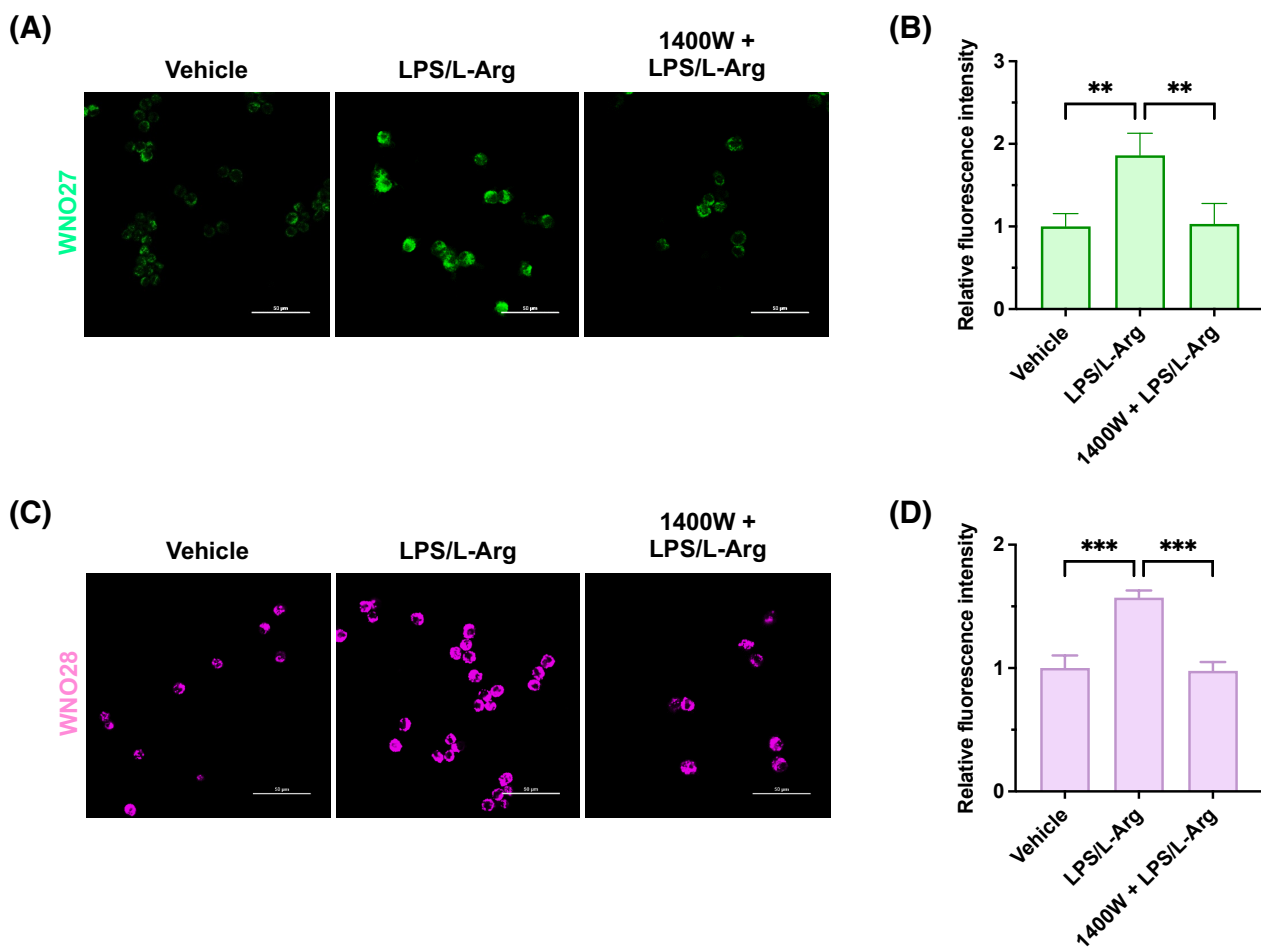


Figure S12. Confocal fluorescence imaging of endogenous NO with WNO27 and WNO28. (A) RAW264.7 cells were treated with LPS (20 $\mu\text{g}/\text{mL}$) and L-Arg (5 mg/mL) in the absence or presence of 1400W (100 μM) for 12 h and loaded with WNO 27 (2 μM) for 60 min. (B) Relative fluorescence intensities of fluorescence images shown in (A). (C) RAW264.7 cells were treated with LPS (20 $\mu\text{g}/\text{mL}$) and L-Arg (5 mg/mL) in the absence or presence of 1400W (100 μM) for 12 h and loaded with WNO 28 (1 μM) for 60 min. (D) Relative fluorescence intensities of fluorescence images shown in (C). Scale bars are 50 μm . Data in (B) and (D) are shown as mean \pm standard deviation ($n = 3$). Statistical analyses were performed using one-way ANOVA. ** $p < 0.01$, *** $p < 0.001$.

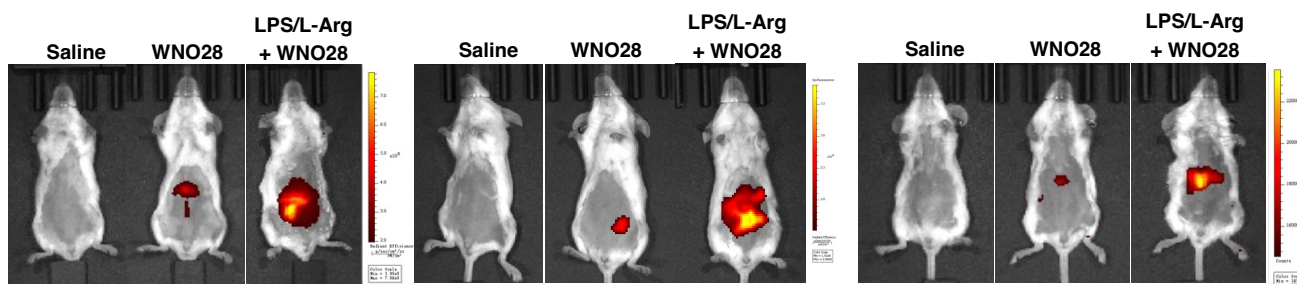


Figure S13. Three replicates of *in vivo* fluorescence imaging of endogenous NO using WNO28. Normal and LPS/L-Arg-stimulated mice were intravenously injected with WNO28 (250 μ M, 200 μ L) or saline and imaged. Related to Figures 7A and 7B.

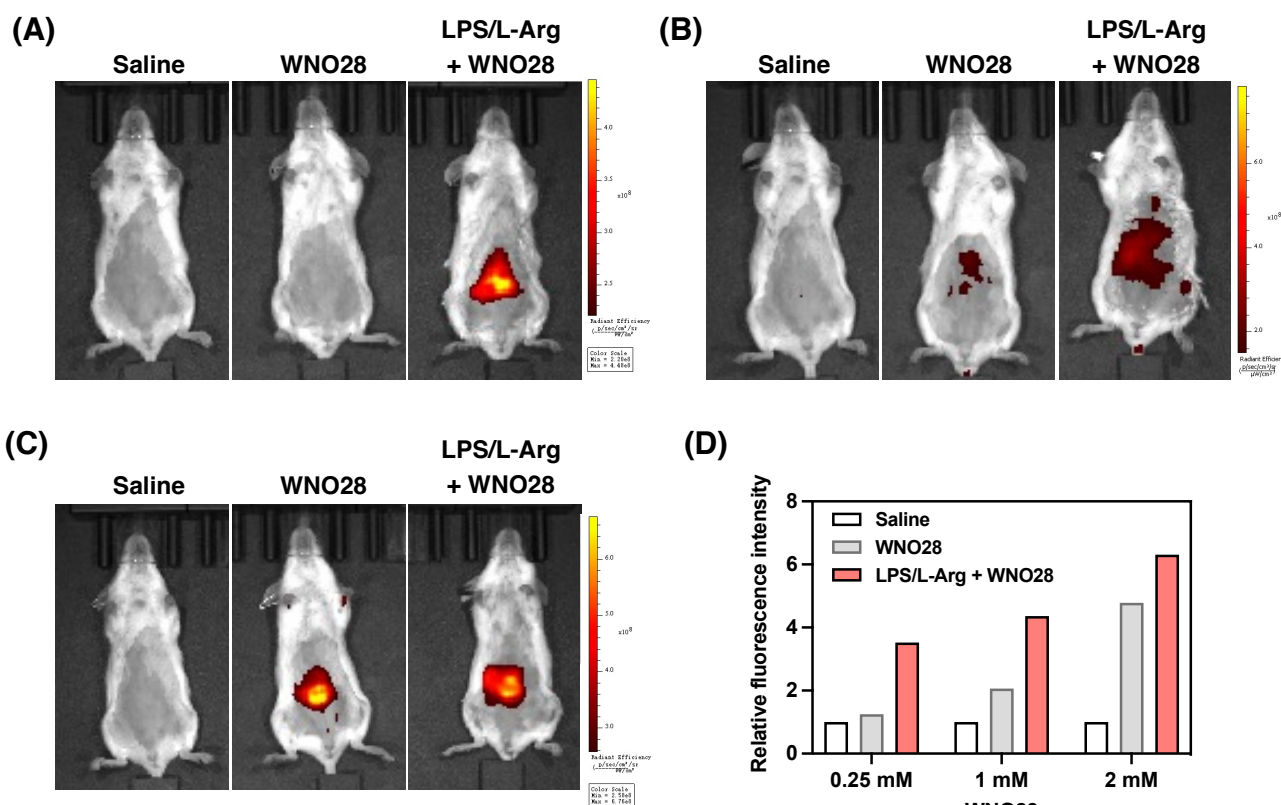


Figure S14. *In vivo* imaging of endogenous NO with WNO28 in inflammatory mice. Normal and LPS/L-Arg-stimulated mice were intravenously injected with either 200 μ L of saline or WNO28 at a dose of (A) 1.89 mg/kg (250 μ M, 200 μ L), (B) 7.56 mg/kg (1 mM, 200 μ L), (C) 15.12 mg/kg (2 mM, 200 μ L), and imaged after 1.5 h. (D) Quantification of fluorescence images shown in (A–C). The y-axis represents fluorescence intensities of mice relative to those without injection of WNO28.

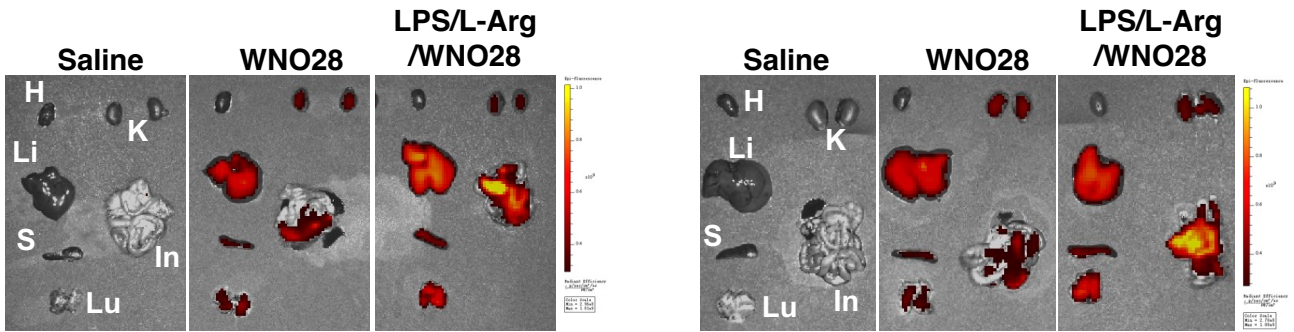


Figure S15. Another two replicates of fluorescence imaging of major organs harvested from normal and LPS/L-Arg-stimulated mice receiving WNO28. Related to Figures 7C and 7D. H: heart, Li: liver, S: spleen, Lu: lung, K: kidneys, In: intestine.

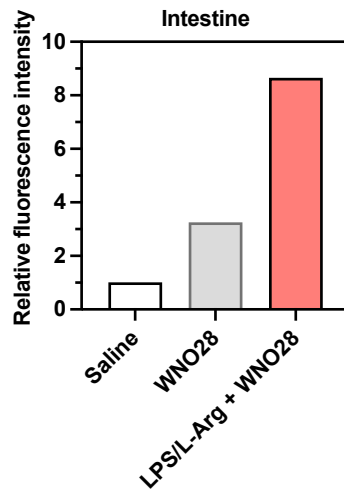


Figure S16. *Ex vivo* relative fluorescence intensities of harvested intestines shown in Figures 7C and 7D.

Experimental procedures

Photophysical characterization of probes

For photophysical characterization, the fluorescent probes were dissolved in DMSO to make the stock solutions at 20 mM. All photophysical and spectroscopic measurements were performed in 20 mM PBS at pH 7.4. Specifically, the probe stock solutions were diluted to 10 μ M as the testing solutions with PBS buffer (containing 0.1% DMSO). Fluorescence spectra and intensities were recorded on a Hitachi F-4600 fluorescence spectrophotometer or a Cytation 5 Multi-Mode Reader (BioTek). Slit widths were generally set at 2.5 nm for both excitation and emission spectra, and the photomultiplier voltage was 700 V. Samples were in 1 cm \times 1 cm quartz cuvettes for measurement.

Diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate (DEA NONOate)¹ was purchased from Sigma. NO was generated from a solution of DEA NONOate in degassed PBS buffer. The NO concentration was measured with the Griess reagents (Beyotime, S0021M). To test the fluorescence responses of fluorescent probes toward NO, aliquots of the NO solution were slowly added into the probe solutions (10 μ M in PBS, 5 mL) with vigorously stirring at room temperature. The volume changes after addition of the NO solutions were less than 1%. The mixtures were incubated at 37°C for 1 h before measurement of the fluorescence spectra and intensities. The limit of detection (LOD) was calculated by $3\sigma/k$, where σ was the standard deviation of blank measurement and k was the slope of the linear relationship between the fluorescence intensity and NO concentration.

For selectivity assays, the testing solution of the probe in PBS (10 μ M; 5 mL) was treated with the analyte of interest (25 μ L of the stock solution) at 37 °C for 1 h. Sources of reactive species (NaClO, H₂O₂, O₂⁻, ¹O₂, [•]OH, and ONOO⁻) for selectivity assays were described as previously.^{2,3} NaNO₂, Cys, GSH, MGO, DHA, and AA were obtained from commercial sources and dissolved or diluted in water (100 mM stock solutions). All analytes were present in 10-fold excess relative to the probe.

LC-MS analysis on reactions of fluorescent probes with NO

To a solution of the probe (0.25 mM) in a mixed solvent of PBS and acetonitrile (v/v = 1:1) was added DEA NONOate solution (final 5 mM) at room temperature with rigorous stirring. The resulting

mixture was incubated at 37 °C for 0.5 h and then analyzed by HPLC-MS on a Q Exactive Focus LC-MS/MS System (ThermoFisher). The UV detector was set at 254 nm, 440 nm, 492 nm, and 670 nm. Samples were loaded onto a Hypersil GOLD VANQUISH C18 UHPLC column (100 × 2.1 mm, 1.9 μm) and eluted with a linear gradient from 10% acetonitrile/90% water containing 0.1 % FA to 95% acetonitrile/5% water containing 0.1 % FA at a flow rate of 0.28 mL/min. HPLC and mass spectra were analyzed with the Xcalibur software (ThermoFisher).

Cell culture

HeLa and RAW264.7 cells were purchased from the American Type Culture Collection (ATCC) and grown in DMEM (Dulbecco's modified Eagle's medium; Corning, 10-013-CVR) supplemented with 10% FBS (fetal bovine serum; Corning, 35-076-CV) at 37 °C in a humidified incubator with an atmosphere of 5% CO₂.

Confocal fluorescence imaging of NO in live cells

For fluorescence imaging of exogenous NO, HeLa cells were seeded on 35-mm cover-slip dishes (MatTek, P35G-1.5-10-C). After overnight culture, cells were washed twice with PBS and incubated with WNO27 (5 μM) or WNO28 (2 μM) in PBS for 30 min at 37 °C/5% CO₂. After washing with PBS, cells were treated with varying concentrations of DEA NONOate in PBS for 60 min and washed with PBS three times. Cells were then imaged in FluoroBrite DMEM (Thermo Fisher Scientific) on a Nikon A1R confocal fluorescence microscope with a 20x objective lens. For co-localization experiments of WNO27, cells were stained with Hoechst 33342, Mito-Tracker Deep Red FM (Beyotime; C1032), or FluoLyso™ Deep Red (Uelandy; L4087S). For co-localization experiments of WNO28, cells were stained with Hoechst 33342, Mito-Tracker Red CMXRos (Beyotime; C1049B), or Lyso-Tracker Red (Beyotime; C1046). For staining endoplasmic reticulum (ER), HeLa cells expressing ER-localizing mCherry were used. Cells were imaged in FluoroBrite DMEM on a confocal fluorescence microscope (Nikon A1R) equipped with a 100x oil immersion objective.

For fluorescence imaging of endogenous NO, RAW264.7 cells were seeded on 35-mm cover-

slip dishes (MatTek, P35G-1.5-10-C). After overnight culture, cells were pre-treated with LPS (20 µg/mL) and L-Arg (5 mg/mL) for 12 h and incubated with WNO27 (2 µM) or WNO28 (1 µM) in PBS for 60 min at 37 °C/5% CO₂. Cells were then washed with PBS three times, stained with Hoechst 33342 (Thermo Fisher Scientific), and imaged in FluoroBrite DMEM (Thermo Fisher Scientific) on a Nikon A1R confocal fluorescence microscope with a 100x objective lens. For inhibitor treatment, cells were pretreated with AG (0.5 mM) or 1400W (100 µM) for 12 h and then treated with LPS and L-Arg as described above.

For confocal fluorescence imaging, Hoechst 33342 was excited with the 405 nm laser, and emission was collected between 425 nm to 475 nm. WNO27 was excited with the 488 nm laser, and emission was collected between 509 nm to 609 nm. WNO28 was excited with the 640 nm laser, and emission was collected between 680 nm to 720 nm. Mito-Tracker Deep Red FM and FluoLysoTM Deep Red were excited with the 640 nm laser, and emission was collected between 690 nm to 720 nm. Mito-Tracker Red CMXRos and ER-Tracker Red was excited with the 561 nm laser, and emission was collected between 570 nm to 600 nm. mCherry was excited with the 561 nm laser, and emission was collected between 620 nm to 680 nm.

Image quantification was performed with the ImageJ software (NIH). At least three fields of view per well were randomly selected for every fluorescence imaging experiment. The fluorescence intensity of every image was quantified in ImageJ and averaged to be the mean fluorescence intensity of the well. For each condition, multiple wells (reported as *n*) were analyzed. The mean fluorescence intensities of these wells were grouped for statistical analysis. Statistical analyses for multiple comparisons were performed in GraphPad Prism using methods indicated in the figure captions.

MTT assay

MTT assay was performed to examine the cytotoxicity of WNO27 and WNO28. Briefly, HeLa cells were incubated with individual probes for 24 h and washed with PBS. After that, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solutions (Beyotime, C0009) were added and incubated with the cells for 4 h at 37 °C. Formazan solubilization solutions were then added and incubated with the cells for another 4 h at 37 °C. The absorbance at 570 nm was measured

with a Cytation 5 plate reader.

Fluorescence imaging of NO in living mice

Animal experiments were performed in strict accordance with guidelines in the *Guide for the Care and Use of Laboratory Animals* (Eighth Edition, National Research Council, 2011, Washington, DC: The National Academies Press) and were approved by the Institutional Animal Care and Use Committee of Peking University Shenzhen Graduate School (Shenzhen, China). The female Balb/c mice (6–8 weeks old, 18–20 g) was purchased from Guangdong Medical Laboratory Animal Center. Prior to experiments, hairs on the abdominal regions of mice were removed using depilatory cream. For LPS/L-Arg treatment, the mice were intravenously injected with LPS at a dose of 2.5 mg/kg *via* tail vein and intraperitoneally injected with L-Arg at a dose of 5 mg/kg for 24 h. For non-treatment control, the mice were intravenously injected with the same volumes of saline.

In vivo fluorescence imaging was performed using the IVIS Spectrum *in vivo* imaging system (PerkinElmer). Briefly, the mice were anesthetized with inhalation of 2% isoflurane for 30 s in an anaerobic box before imaging. After intravenous injection of WNO28 (7.56 mg/kg; 1 mM) in 0.2 mL saline or the same volume of saline for indicated time periods (e.g., 0, 0.75, 1.5, 2, 3, 24 h), the mice were placed in the IVIS Spectrum imaging system for *in vivo* fluorescence imaging with excitation and emission at 675 nm and 720 ± 10 nm, respectively.

For fluorescence imaging of major organs with WNO28, the mice were euthanized after *in vivo* imaging. Major organs such as livers, lungs, hearts, kidneys, spleens, and intestines were dissected and immediately placed in the IVIS Spectrum imaging system for *ex vivo* fluorescence imaging with excitation and emission at 675 nm and 720 ± 10 nm, respectively.

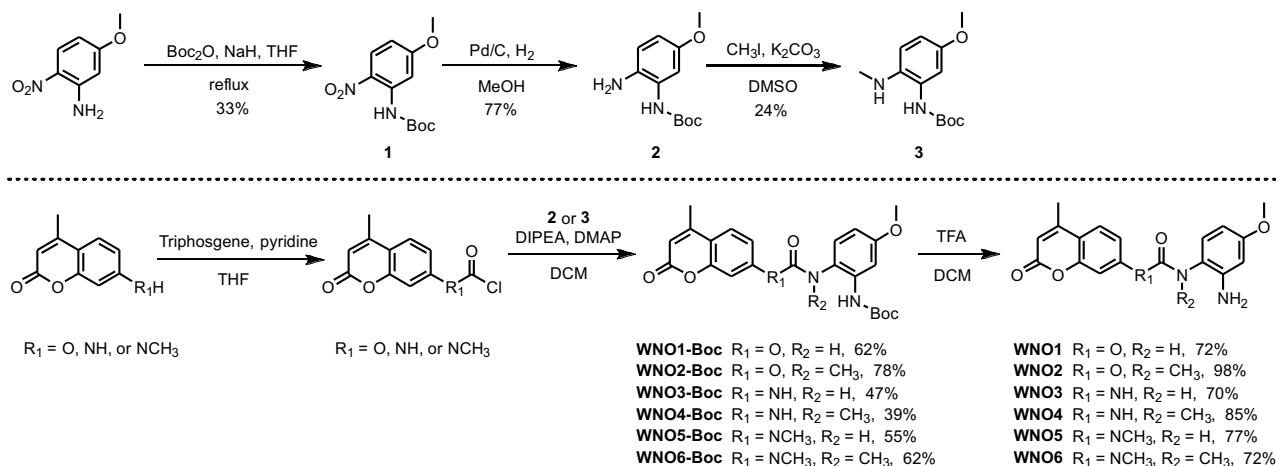
Chemical synthesis

Synthetic materials and methods

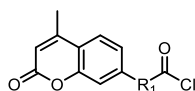
Commercially available chemicals were generally obtained from Sigma-Aldrich, Acros, and TCI, and used as received without further purification. Chemical reactions were performed in oven-dried flasks under a N₂ atmosphere when air- or moisture-sensitive reagents were used. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone. Anhydrous dichloromethane (DCM) was distilled from calcium hydride. Anhydrous dimethylformamide (DMF) was purchased from Sigma-Aldrich. Analytical TLC was conducted on GF₂₅₄ silica gel plates from Qingdao Haiyang Chemical and visualized with a 254 nm UV light and/or staining with iodine (I₂) or phosphomolybdic acid (PMA). Flash column chromatography was performed with silica gel (230-400 mesh, reagent grade) from Qingdao Haiyang Chemical. The ¹H and ¹³C NMR spectra were recorded in CDCl₃, CD₃OD, or *d*⁶-DMSO at room temperature on a Bruker Avance-300 NMR Spectrometer operating at 300 MHz for ¹H, a Bruker Avance-400 NMR Spectrometer operating at 400 MHz for ¹H, a Quantum-I Plus 400 NMR Spectrometer operating at 400 MHz for ¹H, or a Bruker Avance-500 NMR Spectrometer operating at 500 MHz for ¹H. Chemical shifts are reported in δ ppm and *J* values are reported in Hz. ¹H NMR chemical shifts are reported using the residual solvent peaks of CDCl₃ (CD, δ = 7.26 ppm), CD₃OD (CD₃, δ = 3.31 ppm), or *d*⁶-DMSO (D₆, δ = 2.50 ppm) as the internal standards. ¹³C NMR chemical shifts are reported using the residual solvent peaks of CDCl₃ (δ = 77.16 ppm), CD₃OD (δ = 49.00 ppm), or *d*⁶-DMSO (δ = 40.00 ppm) as the internal standards. Splitting patterns are shown as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets. High resolution ESI mass spectra were recorded with an ABI QSTAR Elite or Q Exactive Focus mass spectrometer. LC-MS analysis was performed on a Q Exactive Focus LC-MS/MS System.

Synthesis of probes WNO1–6

Scheme S1. Synthetic scheme for nitric oxide fluorescent probes **WNO1–6**.



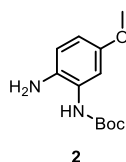
General preparation of coumarin-carbonyl chloride



Coumarin-carbonyl chloride A $R_1 = \text{O}$
Coumarin-carbonyl chloride B $R_1 = \text{NH}$
Coumarin-carbonyl chloride C $R_1 = \text{NCH}_3$

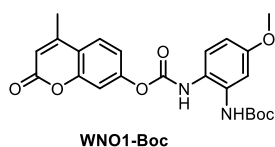
To a solution of 7-hydroxy-4-methyl-2H-chromen-2-one, 7-amino-4-methyl-2H-chromen-2-one, or 4-methyl-7-(methylamino)-2H-chromen-2-one (1 eq) in anhydrous THF was added anhydrous pyridine (0.8 eq) and a solution of triphosgene (0.4 eq) in anhydrous THF. The reaction mixture was stirred at room temperature under a N_2 atmosphere for 4 h. After that, the mixture was concentrated under reduced pressure and vacuumed on an oil pump for 2 h to provide the corresponding carbonyl chloride. The crude product was freshly prepared and used directly for the next step without further purification.

Synthesis of WNO1



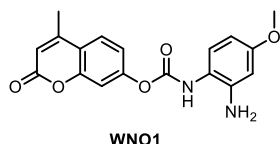
Compound 2. To a solution of 5-methoxy-2-nitroaniline (3.36 g, 20 mmol) in anhydrous THF (200 mL) was added NaH (1.6 g, 40 mmol; 60% in mineral oil) and di-*t*-butyl dicarbonate (Boc_2O ; 8.72 g, 40 mmol). The reaction mixture was heated to reflux overnight. After cooled to room temperature, the

mixture was diluted with H₂O (400 mL) and extracted with ethyl acetate (EA) three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/petroleum ether (PE) = 1:10) to afford compound **1** (1.75 g, 33% yield) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 10.10 (s, 1H), 8.26 – 8.13 (m, 2H), 6.58 (dd, *J* = 9.5, 2.7 Hz, 1H), 3.92 (s, 3H), 1.54 (s, 9H). To a solution of compound **1** in EtOH (25 mL) was added 10% Pd/C (169 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **2** (1.1 g, 77% yield) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.16 (d, *J* = 2.4 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 1H), 6.62 (s, 1H), 6.54 (dd, *J* = 8.6, 2.8 Hz, 1H), 3.74 (s, 3H), 3.28 (s, 2H), 1.51 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 154.3, 153.4, 130.6, 128.1, 120.0, 110.8, 107.9, 80.6, 55.8, 28.4. HRMS calcd for C₁₂H₁₉N₂O₃⁺ [M+H]⁺ 239.1396, found 239.1389.



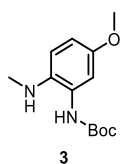
Compound WNO1-Boc. 7-Hydroxy-4-methyl-2H-chromen-2-one (106 mg, 0.6 mmol) was transformed into the corresponding coumarin-carbonyl chloride **A** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added *N,N*-diisopropylethylamine (DIPEA; 0.03 mL, 0.2 mmol), 4-dimethylaminopyridine (DMAP; 12 mg, 0.1 mmol), and compound **2** (47 mg, 0.2 mmol) successively. The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO1-Boc** (55 mg, 62% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 11.06 (s, 1H), 10.55 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.29 (d, *J* = 2.5 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.82 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.80 – 6.70 (m, 2H), 6.15 (t, *J* = 1.3 Hz, 1H), 3.75 (s, 3H), 2.38 (d, *J* = 1.2 Hz, 3H), 1.60 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 161.7,

160.8, 155.3, 155.0, 154.1, 151.5, 148.8, 127.9, 127.2, 122.6, 113.4, 112.5, 110.8, 109.9, 109.9, 102.7, 101.3, 84.2, 56.0, 28.2, 18.6. HRMS calcd for $C_{23}H_{24}N_2NaO_7^+$ $[M+Na]^+$ 463.1481, found 463.1476.



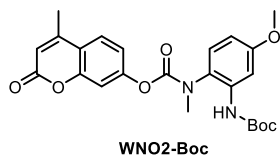
Compound WNO1. To a solution of compound **WNO1-Boc** (40 mg, 0.09 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO1** (22 mg, 72% yield) as a white solid. 1H NMR (400 MHz, d^6 -DMSO) δ 9.28 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.38 (d, J = 2.3 Hz, 1H), 7.30 (dd, J = 8.6, 2.3 Hz, 1H), 7.10 (d, J = 8.7 Hz, 1H), 6.41 (dd, J = 3.8, 2.2 Hz, 2H), 6.28 (dd, J = 8.6, 2.8 Hz, 1H), 4.15 (s, 2H), 3.70 (s, 3H), 2.47 (d, J = 1.3 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.3, 158.7, 156.9, 154.1, 153.6, 152.9, 139.1, 128.0, 126.7, 118.9, 117.4, 116.8, 115.7, 114.4, 113.9, 110.4, 55.5, 18.7. HRMS calcd for $C_{18}H_{17}N_2O_5^+$ $[M+H]^+$ 341.1137, found 341.1136.

Synthesis of WNO2

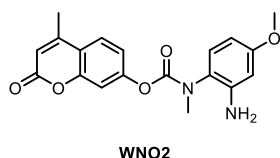


Compound 3. To a solution of compound **2** (1 g, 4.2 mmol) in DMSO (10 mL) was added K_2CO_3 (1.73 g, 12.6 mmol) and iodomethane (0.26 mL, 4.2 mmol). The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with aqueous HCl (1 M) and extracted with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **3** (254 mg, 24% yield) as a white solid. 1H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 1H), 6.74 (d, J = 8.7 Hz, 1H), 6.64 (dd, J = 8.7, 2.9 Hz, 1H), 6.54 (s, 1H), 3.76 (s, 3H), 2.79 (s, 3H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.6, 134.8,

128.0, 115.2, 110.5, 108.4, 80.5, 55.8, 32.5, 28.4. HRMS calcd for $C_{13}H_{21}N_2O_3^+$ $[M+H]^+$ 253.1552, found 253.1547.



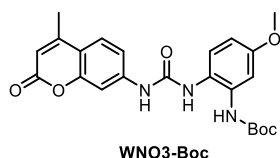
Compound WNO2-Boc. 7-Hydroxy-4-methyl-2H-chromen-2-one (829 mg, 4.71 mmol) was transformed into the corresponding coumarin-carbonyl chloride **A** according to the general procedure. The crude product was dissolved in anhydrous DCM (20 mL). To this solution was added DIPEA (0.26 mL, 1.57 mmol), DMAP (95 mg, 0.78 mmol), and compound **3** (398 mg, 1.57 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (40 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO2-Boc** (559 mg, 78% yield) as a white solid. 1H NMR (400 MHz, d^6 -DMSO) δ 9.15 (s, 1H), 7.78 (d, J = 8.7 Hz, 0.3H), 7.61 (d, J = 8.7 Hz, 0.6H), 7.41 – 7.32 (m, 1H), 7.24 (d, J = 2.9 Hz, 1H), 7.19 (d, J = 2.2 Hz, 1H), 7.11 (dd, J = 8.6, 5.2 Hz, 1H), 6.88 – 6.79 (m, 1H), 6.79 – 6.69 (m, 1H), 6.37 (d, J = 1.4 Hz, 0.3H), 6.15 (d, J = 1.5 Hz, 0.6H), 3.77 (d, J = 3.0 Hz, 3H), 3.28 (s, 2H), 3.16 (s, 1H), 2.44 (d, J = 1.3 Hz, 1H), 2.38 (d, J = 1.2 Hz, 2H), 1.61 (s, 6H), 1.49 (s, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 161.7, 160.8, 158.8, 155.4, 154.1, 150.8, 148.7, 136.3, 129.3, 127.2, 126.6, 124.3, 118.9, 117.5, 113.9, 113.4, 112.5, 110.7, 110.5, 109.8, 109.1, 102.7, 101.3, 84.5, 79.9, 56.1, 55.8, 28.6, 28.1, 27.4, 18.7, 18.6. HRMS calcd for $C_{24}H_{26}N_2NaO_7^+$ $[M+Na]^+$ 477.1638, found 477.1639.



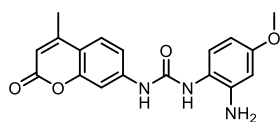
Compound WNO2. To a solution of compound **WNO2-Boc** (200 mg, 0.44 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 2 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product

was recrystallized with PE and DCM to afford compound **WNO2** (154 mg, 98% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 7.83 (d, J = 8.6 Hz, 0.2H), 7.77 (d, J = 8.7 Hz, 0.8H), 7.38 (d, J = 2.3 Hz, 0.2H), 7.32 (dd, J = 8.7, 2.3 Hz, 0.2H), 7.21 (d, J = 2.3 Hz, 0.8H), 7.15 – 7.04 (m, 1.6H), 6.99 (d, J = 8.6 Hz, 0.2H), 6.38 (dd, J = 8.0, 1.4 Hz, 1H), 6.32 (t, J = 2.5 Hz, 1H), 6.15 (dd, J = 8.6, 2.8 Hz, 1H), 3.68 (s, 3H), 3.25 (s, 0.7H), 3.14 (s, 2.3H), 2.45 (dd, J = 9.9, 1.2 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.3, 159.8, 154.7, 153.9, 153.8, 153.6, 145.9, 129.2, 126.6, 120.9, 119.0, 117.4, 113.9, 110.5, 102.6, 100.5, 55.3, 37.5, 18.7. HRMS calcd for $\text{C}_{19}\text{H}_{19}\text{N}_2\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 355.1294, found 355.1285.

Synthesis of WNO3



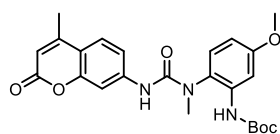
Compound WNO3-Boc. 7-Amino-4-methyl-2H-chromen-2-one (126 mg, 0.72 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.04 mL, 0.24 mmol), DMAP (15 mg, 0.12 mmol), and compound **2** (57 mg, 0.24 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO3-Boc** (50 mg, 47% yield) as a white solid. ^1H NMR (300 MHz, d^6 -DMSO) δ 9.52 (s, 1H), 8.58 (s, 1H), 7.94 (s, 1H), 7.68 (s, 1H), 7.65 – 7.59 (m, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.31 (dd, J = 8.7, 2.1 Hz, 1H), 7.06 (s, 1H), 6.71 (dd, J = 8.9, 2.9 Hz, 1H), 6.27 – 6.16 (m, 1H), 3.72 (s, 3H), 2.39 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, d^6 -DMSO) δ 160.8, 156.5, 154.6, 153.9, 153.9, 153.6, 144.3, 132.6, 126.5, 126.3, 124.3, 114.7, 114.2, 111.9, 110.4, 109.8, 104.6, 80.1, 55.8, 28.6, 18.6. HRMS calcd for $\text{C}_{23}\text{H}_{25}\text{N}_3\text{NaO}_6^+$ $[\text{M}+\text{Na}]^+$ 462.1641, found 462.1635.



WNO3

Compound WNO3. To a solution of compound **WNO3-Boc** (20 mg, 0.05 mmol) in DCM (3 mL) was added trifluoroacetic acid (TFA; 0.2 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO3** (12 mg, 70% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 9.44 (s, 1H), 8.11 (s, 1H), 7.71 – 7.60 (m, 2H), 7.31 (dd, J = 8.7, 2.1 Hz, 1H), 7.14 (d, J = 8.7 Hz, 1H), 6.54 (d, J = 2.8 Hz, 1H), 6.47 (d, J = 8.7 Hz, 1H), 6.19 (d, J = 1.3 Hz, 1H), 3.69 (s, 3H), 2.37 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 165.5, 162.7, 159.3, 158.7, 158.6, 149.1, 132.5, 131.2, 124.8, 122.5, 119.6, 118.9, 116.7, 111.5, 109.5, 109.2, 60.5, 23.3. HRMS calcd for $\text{C}_{18}\text{H}_{18}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 340.1297, found 340.1300.

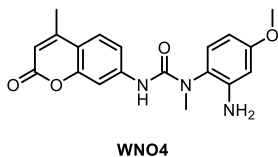
Synthesis of WNO4



WNO4-Boc

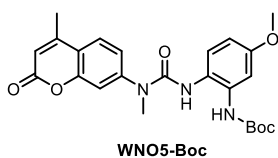
Compound WNO4-Boc. 7-Amino-4-methyl-2H-chromen-2-one (415 mg, 2.37 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.13 mL, 0.79 mmol), DMAP (47 mg, 0.39 mmol), and compound **3** (200 mg, 0.79 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO4-Boc** (138 mg, 39% yield) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.92 (d, J = 2.9 Hz, 1H), 7.51 – 7.39 (m, 2H), 7.23 – 7.11 (m, 2H), 6.80 (s, 1H), 6.68 (dd, J = 8.7, 2.9 Hz, 1H), 6.54 (s, 1H), 6.15 (d, J = 1.3 Hz, 1H), 3.88 (s, 3H), 3.25 (s, 3H), 2.38 (d, J = 1.3 Hz, 3H), 1.50 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3) δ 161.3, 160.8, 154.8, 154.5, 152.5, 152.3, 142.2, 137.2, 129.0, 125.0, 121.8, 115.3, 115.2, 113.1,

110.5, 106.5, 105.0, 81.8, 55.8, 36.8, 28.4, 18.7. HRMS calcd for $C_{24}H_{27}N_3NaO_6^+$ $[M+Na]^+$ 476.1798, found 476.1794.



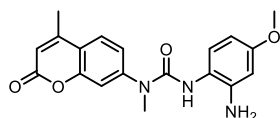
Compound WNO4. To a solution of compound **WNO4-Boc** (52 mg, 0.11 mmol) in DCM (3 mL) was added trifluoroacetic acid (TFA; 0.5 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO4** (33 mg, 85% yield) as a white solid. 1H NMR (400 MHz, d^6 -DMSO) δ 8.16 (s, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.46 (dd, J = 8.8, 2.1 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.49 (d, J = 2.8 Hz, 1H), 6.36 (dd, J = 8.6, 2.8 Hz, 1H), 6.20 (d, J = 1.3 Hz, 1H), 4.61 (s, 2H), 3.74 (s, 3H), 3.11 (s, 3H), 2.39 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.8, 159.9, 159.0, 158.6, 155.0, 154.1, 153.8, 144.6, 130.1, 125.7, 116.1, 114.4, 114.2, 111.9, 106.0, 102.6, 55.5, 36.9, 18.5. HRMS calcd for $C_{19}H_{20}N_3O_4^+$ $[M+H]^+$ 354.1454, found 354.1449.

Synthesis of WNO5



Compound WNO5-Boc. 4-Methyl-7-(methylamino)-2H-chromen-2-one (249 mg, 1.32 mmol) was transformed into the corresponding coumarin-carbonyl chloride **C** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.07 mL, 0.44 mmol), DMAP (26 mg, 0.22 mmol), and compound **2** (104 mg, 0.44 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO5-Boc** (109 mg,

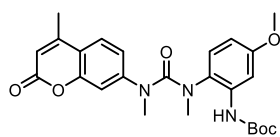
55% yield) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.63 (d, J = 8.4 Hz, 1H), 7.40 – 7.31 (m, 1H), 7.21 (d, J = 8.8 Hz, 1H), 7.08 (s, 1H), 7.05 – 6.93 (m, 2H), 6.64 (dd, J = 8.9, 2.9 Hz, 1H), 6.25 (s, 1H), 3.76 (s, 3H), 3.40 (s, 3H), 2.43 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3) δ 160.5, 157.7, 155.5, 154.4, 154.0, 152.0, 146.7, 133.0, 127.2, 125.9, 123.0, 122.6, 118.4, 114.9, 114.4, 110.9, 108.5, 80.8, 55.6, 37.5, 28.3, 18.8. HRMS calcd for $\text{C}_{24}\text{H}_{27}\text{N}_3\text{NaO}_6^+$ $[\text{M}+\text{H}]^+$ 476.1798, found 476.1794.



WNO5

Compound WNO5. To a solution of compound **WNO5-Boc** (80 mg, 0.18 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.8 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO5** (49 mg, 77% yield) as a white solid. ^1H NMR (300 MHz, CD_3OD) δ 7.83 (d, J = 8.3 Hz, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.25 – 7.14 (m, 1H), 6.91 (d, J = 8.1 Hz, 2H), 6.31 (d, J = 1.4 Hz, 1H), 3.81 (s, 3H), 3.45 (s, 3H), 2.48 (d, J = 1.3 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 161.4, 158.6, 156.4, 154.0, 153.6, 146.5, 129.5, 128.2, 126.1, 124.4, 122.5, 118.4, 114.5, 113.8, 113.4, 108.5, 55.0, 36.9, 17.3. HRMS calcd for $\text{C}_{19}\text{H}_{20}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 354.1454, found 354.1446.

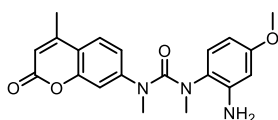
Synthesis of WNO6



WNO6-Boc

Compound WNO6-Boc. 4-Methyl-7-(methylamino)-2H-chromen-2-one (406 mg, 1.62 mmol) was transformed into the corresponding coumarin-carbonyl chloride **C** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.09 mL, 0.54 mmol), DMAP (33 mg, 0.27 mmol), and compound **3** (272 mg, 1.07 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed

with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO6-Boc** (193 mg, 62% yield) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.28 (d, J = 6.7 Hz, 1H), 7.24 (d, J = 2.9 Hz, 1H), 6.80 – 6.71 (m, 3H), 6.39 (dd, J = 8.7, 2.9 Hz, 1H), 6.35 (s, 1H), 6.19 (d, J = 1.4 Hz, 1H), 3.66 (s, 3H), 3.16 (s, 3H), 3.02 (s, 3H), 2.35 (d, J = 1.3 Hz, 3H), 1.51 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3) δ 161.3, 160.5, 159.4, 153.9, 152.0, 151.9, 147.6, 135.3, 127.7, 125.8, 124.7, 121.8, 117.6, 114.4, 113.8, 109.3, 103.6, 81.1, 55.6, 39.8, 38.9, 28.4, 18.7. HRMS calcd for $\text{C}_{25}\text{H}_{29}\text{N}_3\text{NaO}_6^+$ $[\text{M}+\text{Na}]^+$ 490.1954, found 490.1949.

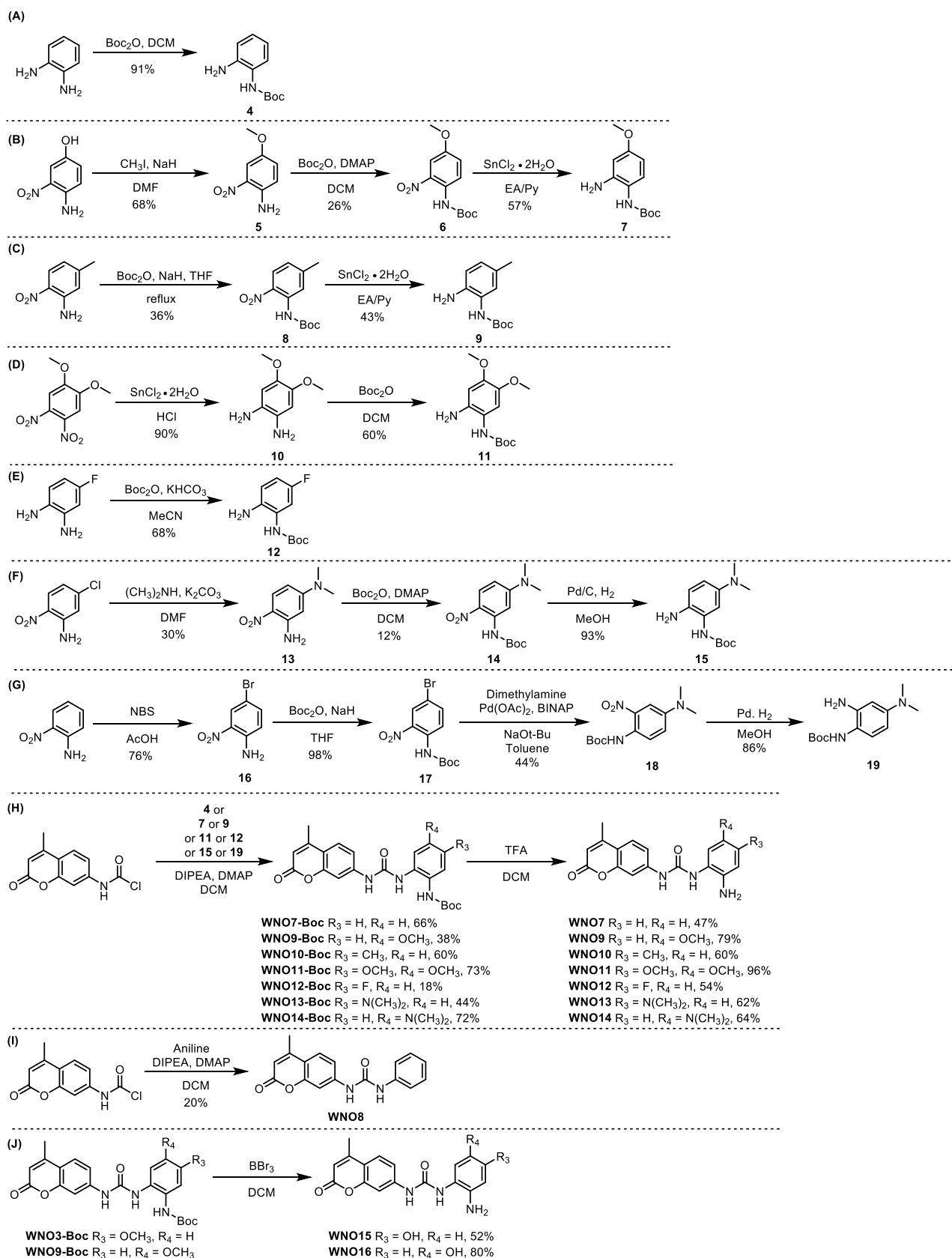


WNO6

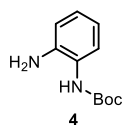
Compound WNO6. To a solution of compound **WNO6-Boc** (120 mg, 0.26 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1.2 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO6** (87 mg, 72%) as a white solid. ^1H NMR (300 MHz, CD_3OD) δ 7.56 (d, J = 8.5 Hz, 1H), 6.98 (dd, J = 8.5, 2.2 Hz, 1H), 6.93 (d, J = 2.1 Hz, 1H), 6.80 (d, J = 8.7 Hz, 1H), 6.33 (dd, J = 8.7, 2.8 Hz, 1H), 6.25 (q, J = 1.3 Hz, 1H), 6.15 (d, J = 2.8 Hz, 1H), 3.63 (s, 3H), 3.19 (s, 3H), 3.03 (s, 3H), 2.42 (d, J = 1.3 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 161.6, 161.4, 159.7, 153.7, 148.2, 139.4, 128.5, 126.2, 125.3, 125.3, 121.0, 117.0, 113.0, 112.1, 106.9, 102.9, 54.6, 38.4, 37.0, 17.3. HRMS calcd for $\text{C}_{20}\text{H}_{22}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 368.1610, found 368.1605.

Synthesis of probes WNO7–16

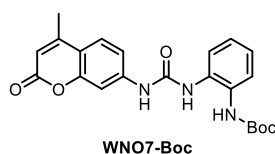
Scheme S2. Synthetic scheme for nitric oxide fluorescent probes WNO7–16.



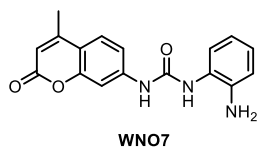
Synthesis of WNO7



Compound 4. To a solution of benzene-1,2-diamine (2.16 g, 20 mmol) in anhydrous DCM (100 mL) was added Boc_2O (872 mg, 4 mmol) slowly. The reaction mixture was stirred at room temperature for 4 h. The mixture was quenched with H_2O (400 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **4** (760 mg, 91%) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.30 (s, 1H), 7.06 – 6.96 (m, 1H), 6.85 – 6.75 (m, 2H), 6.22 (s, 1H), 3.75 (s, 2H), 1.52 (s, 9H).

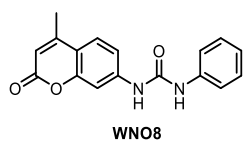


Compound WNO7-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **4** (104 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO7-Boc** (136 mg, 66% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.77 (s, 1H), 8.61 (s, 1H), 8.23 (s, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.66 (d, J = 2.1 Hz, 1H), 7.39 – 7.30 (m, 2H), 7.20 – 7.11 (m, 1H), 7.11 – 7.02 (m, 1H), 6.24 (d, J = 1.3 Hz, 1H), 2.41 (d, J = 1.3 Hz, 3H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 154.5, 154.2, 153.8, 153.0, 144.1, 130.2, 129.7, 126.5, 125.9, 125.6, 123.8, 123.1, 114.7, 114.3, 111.9, 104.6, 79.8, 28.6, 18.5. HRMS calcd for $\text{C}_{22}\text{H}_{23}\text{N}_3\text{NaO}_5^+$ $[\text{M}+\text{Na}]^+$ 432.1535, found 432.1532.



Compound WNO7. To a solution of compound **WNO7-Boc** (40 mg, 0.09 mmol) in DCM (3 mL) was added trifluoroacetic acid (TFA; 0.4 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO7** (13 mg, 47%) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.59 (s, 1H), 8.40 (s, 1H), 7.77 – 7.62 (m, 2H), 7.43 – 7.34 (m, 2H), 7.13 – 7.00 (m, 2H), 6.94 (t, J = 7.6 Hz, 1H), 6.25 (dd, J = 9.3, 1.4 Hz, 1H), 3.64 (s, 2H), 2.42 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 159.1, 158.7, 154.5, 153.8, 153.5, 144.1, 127.7, 126.5, 125.9, 125.5, 119.7, 114.8, 114.3, 112.0, 104.8, 18.5. HRMS calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 310.1192, found 310.1186.

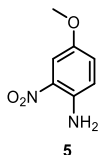
Synthesis of WNO8



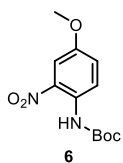
Compound WNO8. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and aniline (0.04 mL, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO8** (30 mg, 20% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.24 (s, 1H), 8.89 (s, 1H), 7.70 (dd, J = 8.7, 6.1 Hz, 1H), 7.64 (d, J = 2.1 Hz, 1H), 7.55 – 7.44 (m, 2H), 7.40 – 7.28 (m, 3H), 7.07 – 6.99 (m, 1H), 6.24 (dd, J = 7.7, 1.4 Hz, 1H), 2.41 (d, J = 1.3 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 154.5, 153.8, 152.7, 143.9, 139.7, 129.4, 126.5, 122.8, 119.0, 114.8, 114.3, 112.0, 104.7, 18.5. HRMS calcd for $\text{C}_{17}\text{H}_{15}\text{N}_2\text{O}_3^+$

[M+H]⁺ 295.1083, found 295.1077.

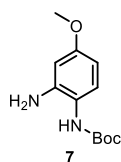
Synthesis of WNO9



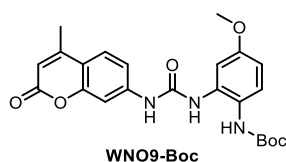
Compound 5. To a solution of 4-amino-3-nitrophenol (4 g, 25.95 mmol) in anhydrous DMF (200 mL) was added NaH (1.04 g, 25.95 mmol; 60% in mineral oil) and iodomethane (1.62 mL, 25.95 mmol). The reaction mixture was stirred at room temperature overnight. After that, the mixture was diluted with H₂O (400 mL) and extracted with ethyl acetate (EA) three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **5** (3.04 g, 68% yield) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 7.49 (d, *J* = 3.0 Hz, 1H), 7.03 (dd, *J* = 9.1, 3.0 Hz, 1H), 6.78 (d, *J* = 9.1 Hz, 1H), 6.11 (s, 2H), 3.76 (s, 3H).



Compound 6. To a solution of compound **5** (3.04 g, 17.91 mmol) in anhydrous DCM (200 mL) was added Boc₂O (3.91 g, 17.91 mmol) and DMAP (218 mg, 1.79 mmol) slowly. The reaction mixture was stirred at room temperature overnight. The mixture was quenched with H₂O (400 mL) and extracted with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **6** (1.23 g, 26% yield) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 9.43 (s, 1H), 8.45 (d, *J* = 9.4 Hz, 1H), 7.66 (d, *J* = 3.0 Hz, 1H), 7.23 (dd, *J* = 9.4, 3.0 Hz, 1H), 3.86 (s, 3H), 1.58 (s, 9H).

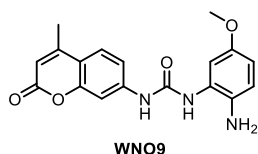


Compound 7. To a solution of compound **6** (759 mg, 2.83 mmol) in EA (60 mL) and pyridine (10 mL) was added stannous chloride dihydrate (3.19 g, 14.15 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was filtered with celite and washed with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **7** (384 mg, 57% yield) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.12 – 7.01 (m, 1H), 6.38 – 6.28 (m, 2H), 6.00 (s, 1H), 3.76 (s, 3H), 1.51 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 158.7, 154.5, 142.7, 127.3, 117.2, 104.5, 102.5, 80.4, 55.4, 28.4. HRMS calcd for C₁₂H₁₉N₂O₃⁺ [M+H]⁺ 239.1396, found 239.1390.



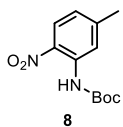
Compound WNO9-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **7** (119 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO9-Boc** (83 mg, 38% yield) as a white solid. ¹H NMR (500 MHz, *d*⁶-DMSO) δ 9.79 (s, 1H), 8.36 (s, 1H), 8.15 (s, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.56 (s, 1H), 7.29 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.10 (s, 1H), 6.64 – 6.51 (m, 1H), 6.21 (d, *J* = 9.0 Hz, 1H), 3.72 (s, 3H), 2.38 (s, 3H), 1.43 (s, 9H). ¹³C NMR (126 MHz, *d*⁶-DMSO) δ 160.6, 157.5, 154.5, 153.7, 152.7, 143.9, 143.4, 128.0, 126.5, 121.4, 115.0, 114.7, 114.3, 112.2, 112.0, 105.1, 104.6, 79.5, 55.7, 28.6, 18.5. HRMS calcd for C₂₃H₂₅N₃NaO₆⁺ [M+Na]⁺

462.1641, found 462.1638.

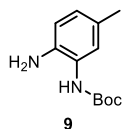


Compound WNO9. To a solution of compound **WNO9-Boc** (28 mg, 0.26 mmol) in DCM (3 mL) was added trifluoroacetic acid (TFA; 0.3 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO9** (16 mg, 79% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.74 (s, 1H), 8.86 (s, 1H), 7.80 – 7.69 (m, 2H), 7.40 (dd, J = 8.7, 2.1 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 7.03 (d, J = 2.8 Hz, 1H), 6.92 (dd, J = 8.8, 2.8 Hz, 1H), 6.27 (d, J = 1.4 Hz, 1H), 3.80 (s, 3H), 2.43 (d, J = 1.3 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.6, 159.4, 154.4, 153.8, 153.5, 143.5, 133.4, 126.5, 125.6, 119.2, 115.1, 114.7, 112.3, 112.0, 111.3, 105.3, 56.1, 18.5. HRMS calcd for $\text{C}_{18}\text{H}_{18}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 340.1297, found 340.1292.

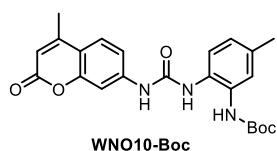
Synthesis of WNO10



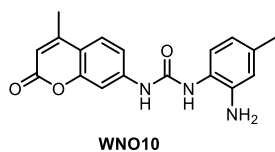
Compound 8. To a solution of 5-methyl-2-nitroaniline (1.5 g, 10 mmol) in anhydrous THF (100 mL) was added NaH (0.4 g, 10 mmol; 60% in mineral oil) and Boc₂O (2.18 g, 10 mmol). The reaction mixture was heated to reflux overnight. After cooled to room temperature, the mixture was diluted with H₂O (200 mL) and extracted with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **8** (0.9 g, 36% yield) as a yellow solid. ^1H NMR (300 MHz, CDCl₃) δ 9.79 (s, 1H), 8.42 (s, 1H), 8.11 (dd, J = 8.7, 3.2 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 2.44 (s, 3H), 1.57 (s, 9H).



Compound 9. To a solution of compound **8** (0.9 g, 3.6 mmol) in EA (60 mL) and pyridine (10 mL) was added stannous chloride dihydrate (4.06 g, 18 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was filtered with celite and washed with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **9** (381 mg, 43% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.21 (s, 1H), 6.84 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.31 (s, 1H), 3.60 (s, 2H), 2.28 (s, 3H), 1.55 (s, 9H).

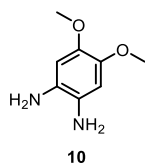


Compound WNO10-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **9** (111 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO10-Boc** (154 mg, 60% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.64 (s, 1H), 8.55 (s, 1H), 8.08 (s, 1H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.33 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.19 (d, *J* = 6.9 Hz, 1H), 6.96 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.24 (dd, *J* = 10.8, 1.3 Hz, 1H), 2.41 (dd, *J* = 3.1, 1.2 Hz, 3H), 2.28 (s, 3H), 1.47 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.7, 154.5, 154.2, 153.8, 153.1, 144.1, 133.2, 129.9, 129.4, 128.7, 126.5, 126.0, 125.8, 114.7, 114.2, 111.9, 104.5, 79.7, 28.6, 21.0, 18.5. HRMS calcd for C₂₃H₂₅N₃NaO₅⁺ [M+Na]⁺ 446.1692, found 446.1686.

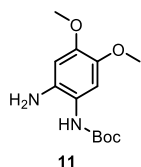


Compound WNO10. To a solution of compound **WNO10-Boc** (50 mg, 0.12 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.5 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO10** (32 mg, 83% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.82 (s, 1H), 8.80 (s, 1H), 7.77 – 7.66 (m, 2H), 7.44 – 7.35 (m, 1H), 7.30 – 7.20 (m, 1H), 7.03 (d, J = 7.1 Hz, 2H), 6.25 (dd, J = 6.0, 1.4 Hz, 1H), 2.42 (d, J = 1.3 Hz, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 159.2, 158.9, 154.5, 153.8, 153.7, 144.0, 135.9, 127.6, 126.5, 126.2, 122.6, 114.9, 114.4, 112.0, 105.0, 21.0, 18.5. HRMS calcd for $\text{C}_{18}\text{H}_{18}\text{N}_3\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 324.1348, found 324.1347.

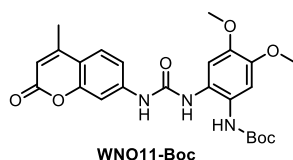
Synthesis of WNO11



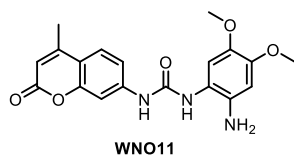
Compound 9. To a solution of 1,2-dimethoxy-4,5-dinitrobenzene (2 g, 8.77 mmol) in ethanol (44 mL) was added stannous chloride dihydrate (19.79 g, 87.7 mmol) and concentrated HCl (35 mL). The reaction mixture was heated to reflux overnight. After cooled to room temperature, the mixture was quenched with 4 M NaOH solution (150 mL) to pH 10. The resulting precipitates were filtered and washed with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:1) to afford compound **10** (1.32 g, 90% yield) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 6.41 (s, 2H), 3.83 (s, 6H), 3.23 (s, 4H).



Compound 11. To a solution of compound **10** (1.32 g, 7.88 mmol) in DCM (50 mL) was added Boc₂O (872 mg, 4 mmol) slowly. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with H₂O (200 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **11** (420 mg, 60% yield) as a brown solid. ¹H NMR (500 MHz, CDCl₃) δ 6.87 (s, 1H), 6.35 (s, 1H), 6.27 (s, 1H), 3.80 (d, *J* = 4.1 Hz, 6H), 1.51 (s, 9H).

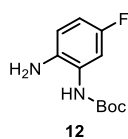


Compound WNO11-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **11** (134 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO11-Boc** (173 mg, 73% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.66 (s, 1H), 8.47 (s, 1H), 8.00 (s, 1H), 7.70 (s, 1H), 7.64 (d, *J* = 2.1 Hz, 1H), 7.34 (dd, *J* = 8.7, 2.1 Hz, 2H), 6.93 (s, 1H), 6.23 (d, *J* = 1.4 Hz, 1H), 3.74 (d, *J* = 6.7 Hz, 6H), 2.41 (d, *J* = 1.3 Hz, 3H), 1.47 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 161.1, 160.7, 154.5, 154.3, 153.8, 153.2, 146.9, 146.4, 145.7, 144.2, 135.9, 126.5, 114.7, 114.2, 111.9, 110.2, 104.5, 79.6, 56.3, 56.2, 28.6, 18.5. HRMS calcd for C₂₄H₂₇N₃NaO₇⁺ [M+Na]⁺ 492.1747, found 492.1742.

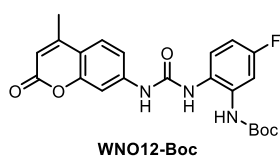


Compound WNO11. To a solution of compound **WNO11-Boc** (50 mg, 0.12 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.5 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO10** (39 mg, 96% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.84 (s, 1H), 8.79 (s, 1H), 7.72 (dd, $J = 5.4, 3.3$ Hz, 2H), 7.39 (dd, $J = 8.7, 2.1$ Hz, 1H), 6.99 (s, 1H), 6.92 (s, 1H), 6.25 (d, $J = 1.4$ Hz, 1H), 4.18 (s, 2H), 3.79 (d, $J = 2.5$ Hz, 7H), 2.42 (d, $J = 1.2$ Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 159.1, 158.8, 154.5, 153.8, 148.0, 147.2, 143.9, 126.5, 124.2, 115.0, 114.5, 112.1, 110.6, 107.2, 105.1, 56.4, 18.5. HRMS calcd for $\text{C}_{19}\text{H}_{20}\text{N}_3\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 370.1403, found 370.1403.

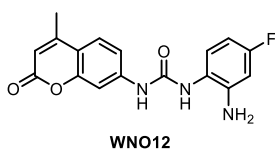
Synthesis of WNO12



Compound 12. To a solution of 4-fluorobenzene-1,2-diamine (1.26 g, 10 mmol) in acetonitrile (100 mL) was added KHCO_3 (2 g, 20 mmol) and Boc_2O (872 mg, 4 mmol) slowly. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with H_2O (200 mL) and extracted with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **12** (615 mg, 68% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 7.11 (dd, $J = 8.3, 5.9$ Hz, 1H), 6.49 – 6.41 (m, 2H), 6.04 (s, 1H), 3.87 (s, 2H), 1.50 (s, 9H).



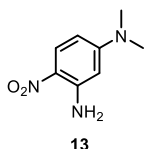
Compound WNO12-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **12** (113 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO12-Boc** (40 mg, 18% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.87 (s, 0.8H), 9.57 (s, 0.2H), 8.78 (s, 0.2H), 8.57 (s, 0.8H), 8.35 (s, 0.8H), 8.11 (s, 0.2H), 7.85 (dd, *J* = 11.5, 3.0 Hz, 0.8H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.64 (t, *J* = 2.3 Hz, 1H), 7.33 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.26 (s, 0.8H), 7.05 – 6.94 (m, 0.2H), 6.93 – 6.80 (m, 0.8H), 6.36 – 6.09 (m, 1H), 2.41 (d, *J* = 1.2 Hz, 3H), 1.59 – 1.36 (m, 9H). ¹⁹F NMR (376 MHz, *d*⁶-DMSO) δ -115.5, -117.9. ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 161.2, 160.7, 160.6, 158.8, 154.5, 153.8, 153.7, 153.3, 152.6, 144.1, 143.6, 135.9, 128.5, 126.6, 126.5, 124.4, 114.8, 114.7, 114.5, 114.2, 112.1, 111.9, 109.5, 109.2, 107.7, 104.7, 104.6, 80.4, 79.8, 28.6, 28.5, 18.5. HRMS calcd for C₂₂H₂₂FN₃NaO₅⁺ [M+Na]⁺ 450.1441, found 450.1438.



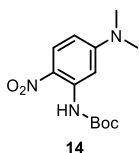
Compound WNO12. To a solution of compound **WNO12-Boc** (40 mg, 0.12 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.4 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO12** (14 mg, 54% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.90 (s, 0.9H), 9.44 (s, 0.1H), 8.82 (s, 0.9H), 8.01 (s, 0.1H), 7.78 – 7.63 (m, 2H), 7.48 – 7.30 (m, 1.9H), 7.23 (dd, *J* = 8.7, 6.2 Hz, 0.1H), 7.12 (dd, *J* = 8.8, 5.7 Hz, 0.9H), 7.04 – 6.89 (m, 0.9H), 6.58 (dd, *J* = 11.0, 2.9 Hz, 0.1H), 6.46 – 6.38 (m, 0.1H), 6.29 – 6.18 (m, 1H), 5.05 (s, 2H), 2.41 (d, *J* = 1.2 Hz, 3H). ¹⁹F NMR (376 MHz, *d*⁶-DMSO) δ -117.5, -119.8. ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.7, 157.1, 154.5, 153.8, 153.1, 143.8, 130.6, 129.4, 126.5, 121.8,

117.9, 114.9, 114.5, 112.1, 111.8, 111.5, 111.2, 110.9, 105.0, 104.5, 18.5. HRMS calcd for $C_{17}H_{15}FN_3O_3^+$ $[M+H]^+$ 328.1097, found 328.1091.

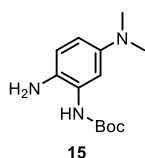
Synthesis of WNO13



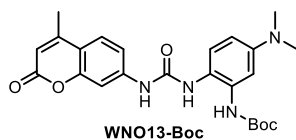
Compound 13. To a solution of 5-chloro-2-nitroaniline (4 g, 23.2 mmol) in DMF (50 mL) was added K_2CO_3 (5.76 g, 41.7 mmol) and dimethylamine solution (33%; 8.8 mL, 58.0 mmol). The reaction mixture was heated to 150 °C for 12 h. After that, the mixture was cooled to room temperature and poured into iced water. The resulting precipitates were filtered and washed with water. The residue was purified by silica gel column chromatography (EA/PE = 1:1) to afford compound **13** (1.25 g, 30% yield) as a yellow solid. 1H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 9.7 Hz, 1H), 6.11 (dd, J = 9.7, 2.6 Hz, 1H), 5.73 (d, J = 2.6 Hz, 1H), 3.03 (s, 6H).



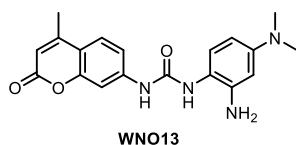
Compound 14. To a solution of compound **13** (1.25 g, 6.9 mmol) in DCM (20 mL) was added DMAP (40 mg, 0.32 mmol) and Boc_2O (2.26 g, 10.4 mmol) slowly. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with H_2O (200 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:3) to afford compound **14** (236 mg, 12% yield) as a yellow solid. 1H NMR (300 MHz, $CDCl_3$) δ 8.13 (dd, J = 9.4, 1.1 Hz, 1H), 6.57 (dd, J = 9.5, 2.8 Hz, 1H), 6.39 (d, J = 2.8 Hz, 1H), 3.09 (d, J = 1.2 Hz, 6H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 152.7, 138.9, 128.7, 125.2, 105.7, 99.2, 81.3, 40.3, 28.3. HRMS calcd for $C_{13}H_{20}N_3O_4^+$ $[M+H]^+$ 282.1454, found 282.1449.



Compound 15. To a solution of compound **14** (154 mg, 0.55 mmol) in MeOH (15 mL) was added 10% Pd/C (15 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:3) to afford compound **15** (129 mg, 93% yield) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.05 (d, *J* = 2.8 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.66 (s, 1H), 6.47 (dd, *J* = 8.6, 2.8 Hz, 1H), 3.22 (s, 2H), 2.86 (s, 6H), 1.53 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 153.5, 146.4, 128.4, 128.2, 120.4, 110.8, 108.4, 80.3, 41.8, 28.4. HRMS calcd for C₁₃H₂₂N₃O₂⁺ [M+H]⁺ 252.1712, found 252.1720.

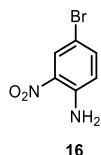


Compound WNO13-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **15** (129 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO13-Boc** (100 mg, 44% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.49 (s, 1H), 8.46 (s, 1H), 7.81 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.37 – 7.27 (m, 2H), 6.89 – 6.80 (m, 1H), 6.55 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.22 (d, *J* = 1.4 Hz, 1H), 2.88 (s, 6H), 2.41 (d, *J* = 1.3 Hz, 3H), 1.47 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.7, 154.5, 153.9, 153.8, 153.7, 148.5, 144.5, 137.4, 132.6, 126.4, 114.6, 114.0, 111.7, 109.6, 108.3, 104.4, 102.1, 79.7, 40.9, 28.6, 18.5. HRMS calcd for C₂₄H₂₈N₄NaO₅⁺ [M+Na]⁺ 475.1957, found 475.1955.

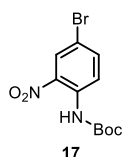


Compound WNO13. To a solution of compound **WNO13-Boc** (50 mg, 0.11 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.5 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO13** (24 mg, 62% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.99 (s, 1H), 8.71 (s, 1H), 7.77 – 7.61 (m, 2H), 7.35 (dd, J = 8.7, 2.1 Hz, 1H), 7.17 (d, J = 8.6 Hz, 1H), 6.56 (d, J = 2.7 Hz, 1H), 6.50 (dd, J = 8.8, 2.7 Hz, 1H), 6.27 – 6.16 (m, 1H), 4.02 (s, 2H), 2.94 (s, 6H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.8, 154.5, 154.0, 153.9, 147.0, 144.6, 138.4, 127.0, 126.4, 119.2, 115.0, 114.7, 114.0, 111.7, 107.3, 104.5, 42.3, 18.5. HRMS calcd for $\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 353.1614, found 353.1607.

Synthesis of WNO14

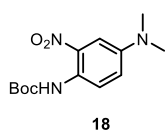


Compound 16. To a solution of 2-nitroaniline (2.76 g, 20 mmol) in acetic acid (8 mL) was added *N*-bromosuccinimide (NBS; 3.56 g, 20 mmol). The reaction mixture was heated to 50 °C for 3 h. After that, the mixture was cooled to room temperature and poured into iced water. The resulting precipitates were filtered and washed with water. The residue was recrystallized with EtOH to afford compound **16** (3.3 g, 76%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 8.22 (d, J = 2.4 Hz, 1H), 7.39 (dd, J = 8.9, 2.4 Hz, 1H), 6.69 (d, J = 8.9 Hz, 1H), 6.08 (s, 2H).

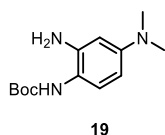


Compound 17. To a solution of compound **16** (3.3 g, 15.34 mmol) in anhydrous THF (200 mL) was

added NaH (1.22 g, 30.7 mmol; 60% in mineral oil) and Boc₂O (3.34 g, 15.34 mmol). The reaction mixture was heated to reflux overnight. After cooled to room temperature, the mixture was diluted with H₂O (200 mL) and extracted with EA three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **17** (4.75 g, 98% yield) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 9.57 (s, 1H), 8.48 (d, *J* = 9.1 Hz, 1H), 8.30 (d, *J* = 2.4 Hz, 1H), 7.65 (dd, *J* = 9.2, 2.4 Hz, 1H), 1.50 (s, 9H).

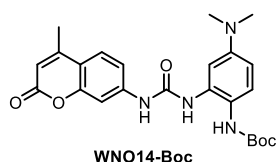


Compound 18. To a Schlenk flask was added Pd(OAc)₂ (45 mg, 0.2 mmol), BINAP (187 mg, 0.3 mmol), sodium tert-butoxide (134 mg, 1.4 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (316 mg, 1 mmol) in anhydrous toluene (10 mL) and dimethylamine (0.9 mL, 1.8 mmol; 2 M in THF solution). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **18** (136 mg, 44% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 9.21 (s, 1H), 8.32 (d, *J* = 9.4 Hz, 1H), 7.42 (d, *J* = 3.0 Hz, 1H), 7.07 (dd, *J* = 9.4, 3.0 Hz, 1H), 3.01 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 152.8, 145.8, 137.4, 125.6, 122.5, 120.6, 107.1, 81.1, 40.6, 28.3. HRMS calcd for C₁₃H₁₉N₃NaO₄⁺ [M+Na]⁺ 304.1273, found 304.1267.

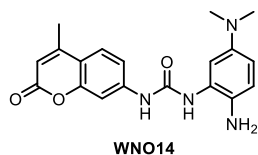


Compound 19. To a solution of compound **18** (136 mg, 0.44 mmol) in MeOH (10 mL) was added 10% Pd/C (14 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:1) to afford

compound **19** (95 mg, 86% yield) as a yellowish oil. ^1H NMR (400 MHz, CDCl_3) δ 7.01 (d, J = 8.7 Hz, 1H), 6.22 (dd, J = 8.7, 2.7 Hz, 1H), 6.16 (d, J = 2.7 Hz, 1H), 5.98 (s, 1H), 2.92 (s, 6H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 150.2, 142.4, 129.9, 127.3, 114.0, 104.5, 101.3, 80.1, 40.9, 28.4. HRMS calcd for $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_2^+$ $[\text{M}+\text{H}]^+$ 252.1712, found 252.1705.



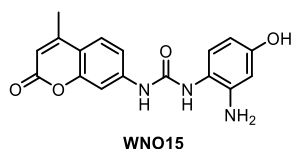
Compound WNO14-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **19** (125 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO14-Boc** (162 mg, 72% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.79 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 7.74 – 7.62 (m, 2H), 7.31 (dd, J = 8.8, 2.0 Hz, 2H), 7.02 (s, 1H), 6.51 – 6.37 (m, 1H), 6.22 (d, J = 1.4 Hz, 1H), 2.88 (s, 6H), 2.40 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.8, 155.0, 154.5, 153.9, 152.9, 149.2, 144.0, 134.8, 127.8, 126.7, 126.5, 118.2, 114.7, 114.2, 111.9, 107.9, 104.6, 79.3, 40.9, 28.6, 18.5. HRMS calcd for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{NaO}_5^+$ $[\text{M}+\text{Na}]^+$ 475.1957, found 475.1954.



Compound WNO14. To a solution of compound **WNO14-Boc** (66 mg, 0.11 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.6 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO14** (34 mg, 64% yield) as a

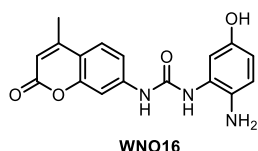
white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.88 (s, 1H), 8.76 (s, 1H), 7.71 (dd, J = 5.4, 3.3 Hz, 2H), 7.38 (dd, J = 8.7, 2.1 Hz, 1H), 7.00 (s, 2H), 6.70 (d, J = 9.2 Hz, 1H), 6.24 (d, J = 1.5 Hz, 1H), 2.91 (s, 6H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 154.5, 153.8, 153.5, 144.1, 142.8, 140.8, 138.8, 133.9, 132.7, 126.5, 126.2, 114.9, 114.3, 112.0, 104.8, 42.3, 18.5. HRMS calcd for $\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 353.1614, found 353.1610.

Synthesis of WNO15



Compound WNO15. To a solution of compound **WNO3-Boc** (174 mg, 0.4 mmol) in anhydrous DCM (10 mL) at $-45\text{ }^\circ\text{C}$ was added BBr_3 (2 mL, 2 mmol; in 1 M CH_2Cl_2 solution). The reaction mixture was stirred at room temperature for 1 h. After completion, the reaction was quenched with water (50 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO15** (73 mg, 52% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.20 (s, 1H), 9.00 (s, 1H), 7.69 – 7.63 (m, 2H), 7.32 (dd, J = 8.7, 2.1 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.22 (dd, J = 11.0, 2.1 Hz, 2H), 6.06 (dd, J = 8.4, 2.7 Hz, 1H), 2.41 (d, J = 1.2 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.8, 156.1, 154.6, 153.9, 153.9, 144.6, 143.6, 127.7, 126.4, 116.0, 114.6, 113.9, 111.7, 104.9, 104.3, 103.0, 18.5. HRMS calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 326.1141, found 326.1144.

Synthesis of WNO16

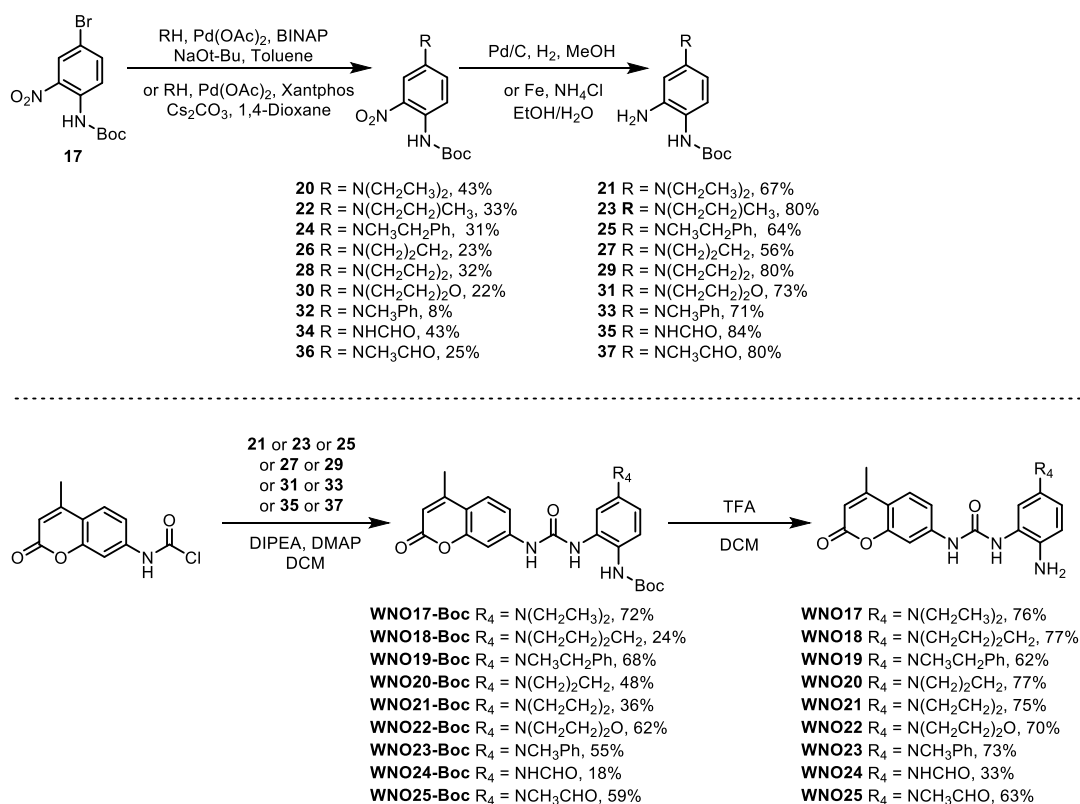


Compound WNO16. To a solution of compound **WNO9-Boc** (100 mg, 0.23 mmol) in anhydrous DCM (10 mL) at $-45\text{ }^\circ\text{C}$ was added BBr_3 (2.3 mL, 2.3 mmol; in 1 M CH_2Cl_2 solution). The reaction mixture was stirred at room temperature for 1 h. After completion, the reaction was quenched with water (50

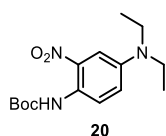
mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO16** (60 mg, 80% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.75 (s, 1H), 9.57 (s, 2H), 8.89 (s, 1H), 7.79 – 7.68 (m, 2H), 7.38 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 2.6 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.26 (s, 1H), 4.88 (s, 2H), 2.42 (s, 3H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.6, 158.0, 154.4, 153.8, 153.5, 143.5, 138.0, 133.3, 126.5, 125.8, 117.0, 115.0, 114.7, 113.4, 112.3, 105.2, 18.5. HRMS calcd for C₁₇H₁₆N₃O₄⁺ [M+H]⁺ 326.1141, found 326.1135.

Synthesis of probes WNO17–25

Scheme S3. Synthetic scheme for nitric oxide fluorescent probes WNO17–25.

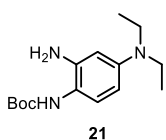


Synthesis of WNO17

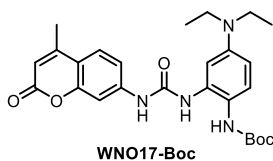


Compound 20. To a Schlenk flask was added Pd(OAc)₂ (45 mg, 0.2 mmol), BINAP (187 mg, 0.3 mmol), sodium tert-butoxide (134 mg, 1.4 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (316 mg, 1 mmol) in anhydrous toluene (10 mL) and diethylamine (0.19 mL, 1.8 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **20** (136 mg, 43% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 9.11 (s, 1H),

8.24 (d, J = 9.4 Hz, 1H), 7.37 (d, J = 3.1 Hz, 1H), 7.00 (dd, J = 9.4, 3.2 Hz, 1H), 3.39 (q, J = 7.1 Hz, 4H), 1.56 (s, 9H), 1.19 (t, J = 7.0 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 152.9, 143.3, 138.0, 124.4, 122.9, 120.0, 106.5, 81.0, 44.5, 28.3, 12.4. HRMS calcd for C₁₅H₂₃N₃NaO₄⁺ [M+Na]⁺ 332.1586, found 332.1580.

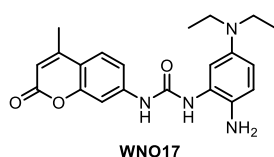


Compound 21. To a solution of compound **20** (136 mg, 0.43 mmol) in MeOH (10 mL) was added 10% Pd/C (14 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:1) to afford compound **21** (82 mg, 67% yield) as a yellowish oil. ¹H NMR (400 MHz, CDCl₃) δ 6.96 (d, J = 8.7 Hz, 1H), 6.15 (dd, J = 8.7, 2.8 Hz, 1H), 6.09 (d, J = 2.8 Hz, 1H), 5.96 (s, 1H), 3.78 (s, 2H), 3.32 (q, J = 7.1 Hz, 4H), 1.53 (s, 9H), 1.16 (t, J = 7.1 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 154.9, 147.4, 142.8, 127.7, 112.7, 103.7, 80.0, 44.5, 28.4, 12.7. HRMS calcd for C₁₅H₂₆N₃O₂⁺ [M+H]⁺ 280.2025, found 280.2019.



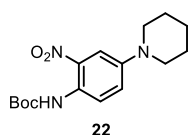
Compound WNO17-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **21** (139 mg, 0.5 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO17-Boc** (172

mg, 72% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.79 (s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 7.77 – 7.61 (m, 2H), 7.31 (dd, J = 9.0, 2.3 Hz, 2H), 7.06 – 6.90 (m, 1H), 6.36 (dd, J = 8.7, 2.9 Hz, 1H), 6.23 (d, J = 1.4 Hz, 1H), 3.32 (q, J = 6.9 Hz, 4H), 2.41 (d, J = 1.2 Hz, 3H), 1.47 (s, 9H), 1.11 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 155.1, 154.5, 153.8, 152.9, 146.2, 144.1, 135.2, 130.2, 128.1, 126.5, 117.0, 114.7, 114.2, 111.9, 107.2, 104.6, 79.2, 44.5, 28.7, 18.5, 13.0. HRMS calcd for $\text{C}_{26}\text{H}_{33}\text{N}_4\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 481.2451, found 481.2449.



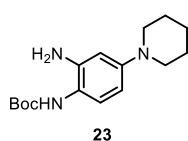
Compound WNO17. To a solution of compound **WNO17-Boc** (49 mg, 0.10 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.4 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO17** (29 mg, 76% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.36 (s, 1H), 7.87 (s, 1H), 7.61 – 7.50 (m, 2H), 7.21 (dd, J = 8.6, 2.2 Hz, 1H), 6.93 (s, 1H), 6.58 (d, J = 8.6 Hz, 1H), 6.26 (d, J = 8.7 Hz, 1H), 6.09 (s, 1H), 4.18 (s, 2H), 3.06 (q, J = 7.1 Hz, 4H), 2.28 (s, 3H), 0.92 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.8, 154.5, 153.8, 153.1, 144.4, 141.2, 131.2, 130.1, 127.1, 126.4, 118.5, 114.6, 114.0, 111.7, 110.3, 104.4, 45.3, 18.5, 12.9. HRMS calcd for $\text{C}_{21}\text{H}_{25}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 381.1927, found 381.1920.

Synthesis of WNO18

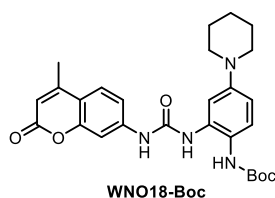


Compound 22. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (45 mg, 0.2 mmol), BINAP (187 mg, 0.3 mmol), sodium tert-butoxide (134 mg, 1.4 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (316 mg, 1 mmol) in anhydrous toluene (10 mL) and piperidine (153 mg, 1.8 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with

celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **22** (106 mg, 33% yield) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 9.35 (s, 1H), 8.36 (d, $J = 9.3$ Hz, 1H), 7.64 (d, $J = 3.0$ Hz, 1H), 7.29 – 7.25 (m, 1H), 3.24 – 3.13 (m, 4H), 1.78 – 1.72 (m, 4H), 1.66 – 1.62 (m, 2H), 1.56 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 147.1, 141.4, 127.8, 125.1, 122.0, 119.0, 111.1, 81.3, 50.5, 28.3, 25.6, 24.0. HRMS calcd for $\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 322.1767, found 322.1762.

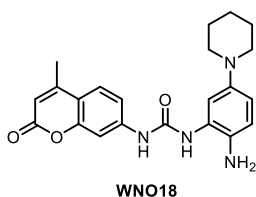


Compound 23. To a solution of compound **22** (106 mg, 0.33 mmol) in MeOH (10 mL) was added 10% Pd/C (11 mg, 10% wt). The reaction mixture was stirred at room temperature under a H_2 atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:1) to afford compound **23** (77 mg, 80% yield) as a yellowish oil. ^1H NMR (400 MHz, CDCl_3) δ 7.04 (d, $J = 8.6$ Hz, 1H), 6.41 (dd, $J = 8.6, 2.7$ Hz, 1H), 6.37 (d, $J = 2.6$ Hz, 1H), 6.04 (s, 1H), 3.79 (s, 2H), 3.16 – 3.06 (m, 4H), 1.77 – 1.65 (m, 6H), 1.62 – 1.56 (m, 2H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 154.5, 151.6, 141.9, 126.7, 116.1, 108.3, 105.3, 80.3, 51.0, 28.4, 25.8, 24.4. HRMS calcd for $\text{C}_{16}\text{H}_{26}\text{N}_3\text{O}_2^+$ $[\text{M}+\text{H}]^+$ 292.2025, found 292.2017.



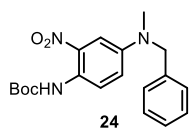
Compound WNO18-Boc. 7-Amino-4-methyl-2H-chromen-2-one (128 mg, 0.54 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.03 mL, 0.18 mmol), DMAP (11 mg, 0.09 mmol), and compound **23** (53 mg, 0.18 mmol) successively. The

reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO18-Boc** (37 mg, 24% yield) as a white solid. ¹H NMR (300 MHz, *d*⁶-DMSO) δ 9.74 (s, 1H), 8.27 (s, 1H), 8.04 (s, 1H), 7.68 (s, 1H), 7.65 – 7.59 (m, 1H), 7.47 (s, 1H), 7.29 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.01 (d, *J* = 8.7 Hz, 1H), 6.59 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.20 (d, *J* = 1.3 Hz, 1H), 3.07 (t, *J* = 5.3 Hz, 4H), 2.38 (d, *J* = 1.2 Hz, 3H), 1.65 – 1.55 (m, 4H), 1.55 – 1.48 (m, 2H), 1.42 (s, 9H). ¹³C NMR (75 MHz, *d*⁶-DMSO) δ 160.7, 154.9, 154.6, 153.8, 153.0, 150.2, 144.1, 132.3, 127.5, 126.6, 120.3, 114.8, 114.3, 112.0, 111.5, 109.7, 104.7, 79.4, 50.5, 28.7, 25.8, 24.5, 18.6. HRMS calcd for C₂₇H₃₃N₄O₅⁺ [M+H]⁺ 493.2451, found 493.2442.

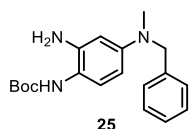


Compound WNO18. To a solution of compound **WNO18-Boc** (20 mg, 0.04 mmol) in DCM (2 mL) was added trifluoroacetic acid (TFA; 0.2 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO18** (12 mg, 77% yield) as a white solid. ¹H NMR (500 MHz, *d*⁶-DMSO) δ 9.39 (s, 1H), 7.89 (s, 1H), 7.68 – 7.58 (m, 2H), 7.31 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.21 – 7.09 (m, 1H), 6.66 (d, *J* = 8.6 Hz, 1H), 6.53 (t, *J* = 5.0 Hz, 1H), 6.19 (q, *J* = 1.2 Hz, 1H), 2.90 (t, *J* = 5.5 Hz, 4H), 2.38 (d, *J* = 1.3 Hz, 3H), 1.69 – 1.56 (m, 4H), 1.50 – 1.43 (m, 2H). ¹³C NMR (126 MHz, *d*⁶-DMSO) δ 160.7, 154.5, 153.7, 153.1, 144.3, 133.8, 126.4, 126.1, 118.0, 117.6, 114.6, 114.5, 114.0, 113.3, 111.8, 104.4, 52.3, 26.1, 24.3, 18.5. HRMS calcd for C₂₂H₂₅N₄O₃⁺ [M+H]⁺ 393.1927, found 393.1921.

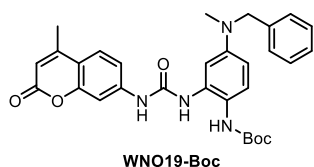
Synthesis of WNO19



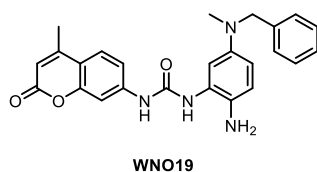
Compound 24. To a Schlenk flask was added Pd(OAc)₂ (45 mg, 0.2 mmol), BINAP (187 mg, 0.3 mmol), sodium tert-butoxide (134 mg, 1.4 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (316 mg, 1 mmol) in anhydrous toluene (10 mL) and *N*-methylbenzylamine (218 mg, 1.8 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **24** (109 mg, 30% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 9.19 (s, 1H), 8.27 (d, *J* = 9.4 Hz, 1H), 7.46 (d, *J* = 3.1 Hz, 1H), 7.39 – 7.32 (m, 2H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.25 – 7.20 (m, 2H), 7.07 (dd, *J* = 9.4, 3.1 Hz, 1H), 4.58 (s, 2H), 3.09 (s, 3H), 1.55 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 152.8, 144.9, 137.8, 137.6, 128.8, 127.3, 126.7, 125.6, 122.6, 120.6, 107.0, 81.2, 56.6, 38.9, 28.3. HRMS calcd for C₁₉H₂₄N₃O₄⁺ [M+H]⁺ 358.1767, found 358.1762.



Compound 25. To a solution of compound **24** (109 mg, 0.31 mmol) in EtOH (5 mL) and THF (2.5 mL) was added iron powder (174 mg, 3.1 mmol) and ammonium chloride (100 mg, 1.8 mmol). The reaction mixture was heated to reflux overnight. After that, the mixture was filtered with celite, washed with EA, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **25** (65 mg, 64% yield) as a yellowish oil. ¹H NMR (500 MHz, CDCl₃) δ 7.33 – 7.28 (m, 2H), 7.28 – 7.18 (m, 3H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.19 (dd, *J* = 8.6, 2.8 Hz, 1H), 6.11 (t, *J* = 2.1 Hz, 1H), 5.96 (s, 1H), 4.48 (s, 2H), 2.96 (d, *J* = 1.6 Hz, 3H), 1.49 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 149.3, 142.6, 139.0, 128.5, 128.5, 128.2, 126.8, 126.8, 113.7, 104.0, 100.7, 80.1, 56.7, 38.6, 28.4. HRMS calcd for C₁₉H₂₆N₃O₂⁺ [M+H]⁺ 328.2025, found 328.2021.



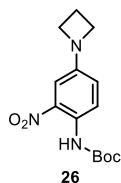
Compound WNO19-Boc. 7-Amino-4-methyl-2H-chromen-2-one (384 mg, 1.62 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.09 mL, 0.54 mmol), DMAP (33 mg, 0.27 mmol), and compound **25** (175 mg, 0.54 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO19-Boc** (195 mg, 68% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.74 (s, 1H), 8.17 (s, 1H), 8.03 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.36 (s, 1H), 7.29 (q, *J* = 6.3, 4.4 Hz, 3H), 7.20 (d, *J* = 8.3 Hz, 3H), 6.95 (s, 1H), 6.47 – 6.34 (m, 1H), 6.19 (s, 1H), 4.52 (s, 2H), 2.96 (s, 3H), 2.37 (s, 3H), 1.42 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.7, 155.0, 154.6, 153.8, 153.0, 148.0, 144.1, 139.5, 130.2, 129.0, 128.0, 127.5, 127.3, 126.5, 118.1, 114.8, 114.3, 112.0, 107.8, 105.7, 104.7, 79.3, 56.1, 39.2, 28.7, 18.6. HRMS calcd for C₃₀H₃₁N₄O₅ [M-H]⁻ 527.2294, found 527.2297.



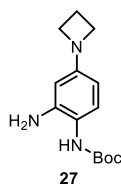
Compound WNO19. To a solution of compound **WNO19-Boc** (195 mg, 0.37 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1.9 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO19** (98 mg, 62% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.92 (s, 1H), 9.07 (s, 1H), 7.79 – 7.67 (m, 2H), 7.43 – 7.30 (m, 3H), 7.30 – 7.19 (m, 3H), 7.12 (d, *J* = 8.5 Hz, 1H), 6.76 (s, 1H), 6.66 (d, *J* = 8.8 Hz, 1H), 6.25 (s, 1H), 4.60 (s, 2H), 3.04 (s, 3H), 2.41 (s, 3H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.7, 154.4, 153.8, 153.7, 145.1, 144.6, 144.1, 143.8, 138.7, 129.0, 127.7, 127.5, 126.5, 122.3, 117.2, 115.2, 115.0, 114.5,

112.1, 105.1, 18.5. HRMS calcd for $C_{25}H_{25}N_4O_3^+$ $[M+H]^+$ 429.1927, found 429.1922.

Synthesis of WNO20

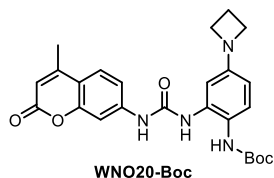


Compound 26. To a Schlenk flask was added $Pd(OAc)_2$ (403 mg, 1.8 mmol), BINAP (1.68 g, 2.7 mmol), sodium tert-butoxide (672 mg, 7 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous toluene (10 mL) and azetidine (513 mg, 9 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **26** (400 mg, 23% yield) as a yellow solid. 1H NMR (500 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.26 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 2.8 Hz, 1H), 6.72 (dd, J = 9.1, 2.8 Hz, 1H), 3.89 (t, J = 7.2 Hz, 4H), 2.54 – 2.33 (m, 2H), 1.52 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7, 147.3, 137.4, 126.2, 122.5, 119.5, 106.2, 81.2, 52.7, 28.3, 16.9. HRMS calcd for $C_{14}H_{19}N_3NaO_4$ $[M+Na]^+$ 316.1273, found 316.1268.

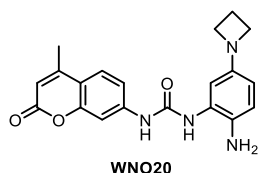


Compound 27. To a solution of compound **26** (200 mg, 0.83 mmol) in MeOH (10 mL) was added 10% Pd/C (20 mg, 10% wt). The reaction mixture was stirred at room temperature under a H_2 atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **27** (123 mg, 56% yield) as a yellow solid. 1H NMR (500 MHz, $CDCl_3$) δ 6.94 (d, J = 8.4 Hz, 1H), 5.95 (s, 1H), 5.88 (dd, J = 8.4, 2.5 Hz, 1H), 5.82 (d, J = 2.5 Hz, 1H), 3.79 (t, J = 7.3 Hz, 4H), 2.36

– 2.45 (m, 2H), 1.49 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 151.9, 142.5, 129.9, 127.4, 114.4, 102.9, 99.7, 80.1, 52.5, 28.4, 16.8. HRMS calcd for $\text{C}_{14}\text{H}_{22}\text{N}_3\text{O}_2^+$ $[\text{M}+\text{H}]^+$ 264.1712, found 264.1716.



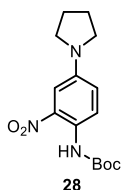
Compound WNO20-Boc. 7-Amino-4-methyl-2H-chromen-2-one (140 mg, 0.59 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (5 mL). To this solution was added DIPEA (0.29 mL, 1.77 mmol), DMAP (36 mg, 0.3 mmol), and compound **27** (156 mg, 0.1 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO20-Boc** (132 mg, 48% yield) as a white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 9.77 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 7.66 (d, $J = 8.7$ Hz, 1H), 7.60 (d, $J = 2.1$ Hz, 1H), 7.29 (dd, $J = 8.7, 2.1$ Hz, 1H), 7.01 (s, 1H), 6.95 (s, 1H), 6.20 (d, $J = 1.3$ Hz, 1H), 6.06 (d, $J = 8.6$ Hz, 1H), 3.75 (t, $J = 7.1$ Hz, 4H), 2.38 (d, $J = 1.2$ Hz, 3H), 2.34 – 2.22 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.7, 155.2, 154.6, 153.7, 152.9, 150.9, 144.1, 130.3, 127.9, 126.6, 118.6, 114.7, 114.3, 112.0, 108.6, 106.4, 104.7, 79.2, 52.6, 28.6, 18.5, 16.8. HRMS calcd for $\text{C}_{25}\text{H}_{28}\text{N}_4\text{NaO}_5^+$ $[\text{M}+\text{Na}]^+$ 487.1957, found 487.1950.



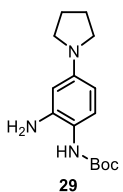
Compound WNO20. To a solution of compound **WNO20-Boc** (132 mg, 0.28 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1.3 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO20** (78 mg, 77% yield) as a

white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 9.78 (s, 1H), 8.58 (s, 1H), 7.68 – 7.64 (m, 2H), 7.33 (dd, J = 8.7, 2.2 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.60 (d, J = 2.5 Hz, 1H), 6.24 – 6.14 (m, 2H), 3.74 (t, J = 7.2 Hz, 4H), 2.38 (d, J = 1.3 Hz, 3H), 2.30 – 2.20 (m, 2H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.6, 154.5, 153.7, 153.3, 148.8, 144.1, 140.0, 126.4, 121.0, 114.8, 114.2, 111.9, 108.9, 108.5, 107.5, 104.7, 53.0, 31.2, 18.5. HRMS calcd for $\text{C}_{20}\text{H}_{21}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 365.1614, found 365.1611.

Synthesis of WNO21

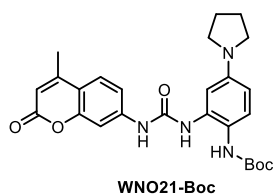


Compound 28. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (403 mg, 1.8 mmol), BINAP (1.68 g, 2.7 mmol), sodium tert-butoxide (672 mg, 7 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous toluene (10 mL) and pyrrolidine (639 mg, 9 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **28** (488 mg, 32% yield) as a yellow solid. ^1H NMR (500 MHz, CDCl_3) δ 9.09 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 7.19 (d, J = 3.0 Hz, 1H), 6.84 (dd, J = 9.2, 3.0 Hz, 1H), 3.38 – 3.17 (m, 4H), 2.13 – 1.96 (m, 4H), 1.52 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 152.8, 143.4, 137.8, 124.4, 122.7, 119.6, 105.9, 80.9, 47.9, 28.3, 25.5. HRMS calcd for $\text{C}_{15}\text{H}_{21}\text{N}_3\text{NaO}_4^+$ $[\text{M}+\text{Na}]^+$ 330.1430, found 330.1424.

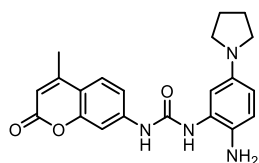


Compound 29. To a solution of compound **28** (488 mg, 1.59 mmol) in MeOH (10 mL) was added 10% Pd/C (49 mg, 10% wt). The reaction mixture was stirred at room temperature under a H_2 atmosphere

overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **29** (354 mg, 80% yield) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 6.94 (d, J = 8.5 Hz, 1H), 6.00 (dd, J = 8.5, 2.6 Hz, 1H), 5.94 (d, J = 2.6 Hz, 1H), 3.76 (s, 2H), 3.37 – 3.10 (m, 4H), 2.10 – 1.88 (m, 4H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 154.9, 147.7, 142.9, 127.8, 112.9, 103.4, 99.8, 80.0, 47.8, 28.5, 25.6. HRMS calcd for $\text{C}_{15}\text{H}_{24}\text{N}_3\text{O}_2^+$ $[\text{M}+\text{H}]^+$ 278.1869, found 278.1860.



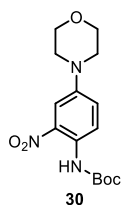
Compound WNO21-Boc. 7-Amino-4-methyl-2H-chromen-2-one (355 mg, 1.5 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (5 mL). To this solution was added DIPEA (0.08 mL, 0.5 mmol), DMAP (30 mg, 0.25 mmol), and compound **29** (354 mg, 1.27 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO21-Boc** (218 mg, 36% yield) as a white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 9.75 (s, 1H), 8.15 (s, 1H), 8.02 (s, 1H), 7.70 – 7.59 (m, 2H), 7.34 – 7.25 (m, 1H), 7.15 (s, 1H), 7.03 – 6.90 (m, 1H), 6.19 (d, J = 10.7 Hz, 2H), 3.19 (d, J = 5.7 Hz, 4H), 2.36 (d, J = 3.0 Hz, 3H), 2.00 – 1.87 (m, 4H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.6, 155.1, 154.5, 153.7, 152.8, 146.6, 144.1, 135.1, 130.1, 128.1, 126.4, 117.1, 114.6, 114.2, 111.9, 107.1, 104.5, 79.7, 79.1, 48.0, 28.6, 25.5, 18.5. HRMS calcd for $\text{C}_{26}\text{H}_{31}\text{N}_4\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 479.2294, found 479.2288.



WNO21

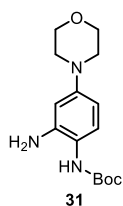
Compound WNO21. To a solution of compound **WNO21-Boc** (218 mg, 0.46 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 2 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO21** (131 mg, 75% yield) as a white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 9.46 (s, 1H), 7.97 (s, 1H), 7.71 – 7.58 (m, 2H), 7.31 (dd, J = 8.7, 2.1 Hz, 1H), 6.85 (s, 1H), 6.69 (d, J = 8.5 Hz, 1H), 6.26 – 6.12 (m, 2H), 3.10 (s, 4H), 2.38 (d, J = 1.2 Hz, 3H), 2.01 – 1.87 (m, 4H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.7, 154.5, 153.8, 153.0, 144.3, 141.6, 136.9, 130.1, 126.4, 119.6, 119.2, 114.6, 114.0, 111.8, 107.3, 104.4, 48.4, 25.3, 18.5. HRMS calcd for $\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 379.1770, found 379.1766.

Synthesis of WNO22

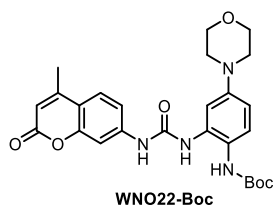


Compound 30. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (403 mg, 1.8 mmol), BINAP (1.68 g, 2.7 mmol), sodium tert-butoxide (672 mg, 7 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous toluene (10 mL) and morpholine (783 mg, 9 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **30** (355 mg, 22% yield) as a yellow solid. ^1H NMR (500 MHz, CDCl_3) δ 9.57 (s, 1H), 8.62 (d, J = 9.3 Hz, 1H), 7.83 (d, J = 3.0 Hz, 1H), 7.45 (dd, J = 9.3, 3.0 Hz, 1H), 4.09 (dd, J = 5.6, 3.9 Hz, 4H), 3.38 (dd, J = 5.8, 3.8 Hz, 4H), 1.76 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 152.6, 146.1, 136.8, 128.7, 124.2, 122.1, 110.7, 81.5, 66.7, 49.2, 28.3. HRMS calcd for $\text{C}_{15}\text{H}_{21}\text{N}_3\text{NaO}_5^+$ $[\text{M}+\text{Na}]^+$ 346.1379,

found 346.1374.

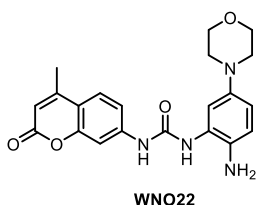


Compound 31. To a solution of compound **30** (335 mg, 1.04 mmol) in MeOH (10 mL) was added 10% Pd/C (34 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **31** (224 mg, 73% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 7.03 (d, *J* = 8.6 Hz, 1H), 6.33 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.28 (d, *J* = 2.7 Hz, 1H), 6.11 (s, 1H), 3.89 – 3.76 (m, 4H), 3.14 – 2.98 (m, 4H), 1.49 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 154.4, 150.5, 142.1, 126.9, 116.9, 107.2, 104.4, 80.3, 66.9, 49.6, 28.4. HRMS calcd for C₁₅H₂₄N₃O₃⁺ [M+H]⁺ 294.1818, found 294.1816.



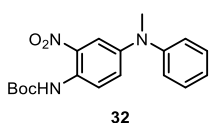
Compound WNO22-Boc. 7-Amino-4-methyl-2H-chromen-2-one (547 mg, 2.31 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (5 mL). To this solution was added DIPEA (0.13 mL, 0.77 mmol), DMAP (48 mg, 0.39 mmol), and compound **31** (224 mg, 0.77 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO22-Boc** (236 mg, 62% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.78 (s, 1H), 8.34 (s, 1H), 8.12 (s, 1H), 7.75 – 7.59 (m, 2H), 7.53 (s, 1H), 7.31 (d, *J* = 8.7 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 1H), 6.65 (d, *J* = 8.9 Hz, 1H), 6.21 (s, 1H), 3.76 (t, *J* = 4.5 Hz, 4H), 3.08 (t, *J* = 3.8 Hz, 4H), 2.40 (s, 3H), 1.45

(s, 9H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 154.8, 154.5, 153.8, 152.9, 149.4, 144.0, 134.5, 127.4, 126.5, 121.0, 114.7, 114.3, 111.9, 110.8, 109.0, 104.6, 79.5, 66.6, 49.3, 28.6, 18.5. HRMS calcd for $\text{C}_{26}\text{H}_{30}\text{N}_4\text{NaO}_6^+ [\text{M}+\text{Na}]^+$ 517.2063, found 517.2057.



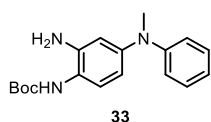
Compound WNO22. To a solution of compound **WNO22-Boc** (140 mg, 0.28 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1.4 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO22** (78 mg, 70% yield) as a white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 10.01 (s, 1H), 8.97 (s, 1H), 7.72 – 7.63 (m, 2H), 7.35 (dd, $J = 8.7, 2.1$ Hz, 1H), 7.09 – 6.97 (m, 2H), 6.78 (dd, $J = 8.8, 2.8$ Hz, 1H), 6.20 (d, $J = 1.4$ Hz, 1H), 3.72 (dd, $J = 6.0, 3.5$ Hz, 4H), 3.06 (t, $J = 4.8$ Hz, 4H), 2.38 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.6, 154.5, 153.7, 153.5, 152.7, 144.0, 130.4, 130.1, 126.4, 122.2, 114.9, 114.3, 113.2, 112.1, 112.0, 104.9, 66.4, 49.6, 18.5. HRMS calcd for $\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}_4^+ [\text{M}+\text{H}]^+$ 395.1719, found 395.1712.

Synthesis of WNO23

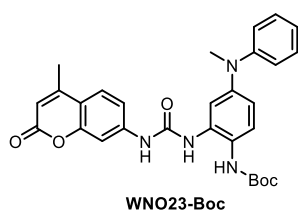


Compound 32. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (224 mg, 1 mmol), BINAP (933 mg, 1.5 mmol), sodium tert-butoxide (672 mg, 7 mmol), and anhydrous toluene (10 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous toluene (10 mL) and *N*-methylaniline (5.35 g, 50 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **32** (144 mg, 8% yield) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 9.34 (s, 1H),

8.30 (d, $J = 9.3$ Hz, 1H), 7.69 (d, $J = 2.9$ Hz, 1H), 7.36 – 7.29 (m, 2H), 7.22 (dd, $J = 9.3, 2.9$ Hz, 1H), 7.10 – 7.02 (m, 3H), 3.33 (s, 3H), 1.53 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 152.7, 148.0, 143.9, 137.1, 129.8, 128.8, 126.9, 123.4, 122.4, 122.1, 113.4, 81.6, 40.5, 28.4. HRMS calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 344.1610, found 344.1606.

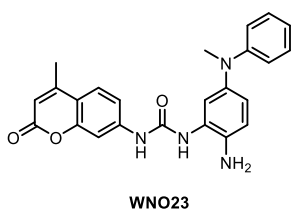


Compound 33. To a solution of compound **32** (119 mg, 0.35 mmol) in EtOH (5 mL) and THF (2.5 mL) was added iron powder (192 mg, 3.5 mmol) and ammonium chloride (93 mg, 1.75 mmol). The reaction mixture was heated to reflux overnight. After that, the mixture was filtered with celite, washed with EA, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **33** (78 mg, 71% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 7.27 – 7.22 (m, 2H), 7.08 (d, $J = 8.4$ Hz, 1H), 7.05 – 6.97 (m, 2H), 6.95 – 6.86 (m, 1H), 6.49 – 6.40 (m, 2H), 6.07 (s, 1H), 3.77 (s, 2H), 3.25 (s, 3H), 1.51 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 149.1, 147.9, 141.8, 129.1, 126.6, 120.9, 120.0, 118.3, 112.3, 109.7, 108.5, 80.4, 40.3, 28.4. HRMS calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_2^+$ $[\text{M}+\text{H}]^+$ 314.1869, found 314.1859.



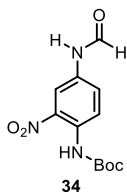
Compound WNO23-Boc. 7-Amino-4-methyl-2H-chromen-2-one (467 mg, 2.01 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (5 mL). To this solution was added DIPEA (0.33 mL, 2.01 mmol), DMAP (41 mg, 0.34 mmol), and compound **33** (211 mg, 0.67 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO23-**

Boc (189 mg, 55% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.72 (s, 1H), 8.42 (s, 1H), 8.14 (s, 1H), 7.65 (d, $J = 8.7$ Hz, 1H), 7.62 – 7.54 (m, 2H), 7.33 – 7.19 (m, 3H), 7.15 (d, $J = 8.7$ Hz, 1H), 7.00 – 6.94 (m, 2H), 6.94 – 6.85 (m, 1H), 6.70 (dd, $J = 8.8, 2.6$ Hz, 1H), 6.19 (d, $J = 1.4$ Hz, 1H), 3.23 (s, 3H), 2.37 (d, $J = 1.3$ Hz, 3H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 160.7, 154.7, 154.6, 153.8, 152.9, 149.2, 146.5, 144.0, 134.5, 129.7, 127.4, 126.6, 123.2, 121.0, 119.6, 116.4, 115.1, 114.8, 114.4, 112.0, 104.7, 79.7, 40.7, 28.7, 18.6. HRMS calcd for $\text{C}_{29}\text{H}_{31}\text{N}_4\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 515.2294, found 515.2206.



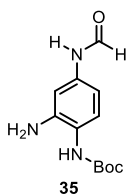
Compound WNO23. To a solution of compound **WNO23-Boc** (189 mg, 0.37 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 1.8 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO23** (112 mg, 73% yield) as a white solid. ^1H NMR (500 MHz, d^6 -DMSO) δ 9.93 (s, 1H), 9.18 (s, 1H), 7.67 (dd, $J = 5.4, 3.3$ Hz, 2H), 7.39 – 7.30 (m, 3H), 7.19 (d, $J = 8.8$ Hz, 1H), 7.11 (d, $J = 7.9$ Hz, 1H), 7.06 (t, $J = 7.4$ Hz, 1H), 6.86 (d, $J = 2.7$ Hz, 1H), 6.76 (dd, $J = 8.8, 2.7$ Hz, 1H), 6.20 (d, $J = 1.5$ Hz, 1H), 3.25 (s, 3H), 2.37 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (126 MHz, d^6 -DMSO) δ 160.6, 154.4, 153.7, 153.6, 148.3, 143.7, 132.5, 130.0, 126.4, 124.7, 123.5, 123.0, 117.4, 115.3, 115.0, 114.5, 114.2, 112.1, 108.0, 105.2, 40.6, 18.5. HRMS calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}_3^+$ $[\text{M}+\text{H}]^+$ 415.1770, found 415.1761.

Synthesis of WNO24

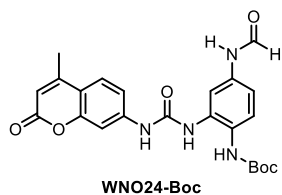


Compound 34. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (224 mg, 1 mmol), Xantphos (867 mg, 1.5 mmol), Cs_2CO_3 (2.43 g, 7.5 mmol), and anhydrous 1,4-dioxane (15 mL). The mixture was stirred at

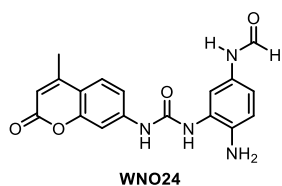
room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous 1,4-dioxane (15 mL) and formamide (270 mg, 6 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **34** (614 mg, 43% yield) as a yellow solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 10.62 – 10.48 (m, 0.8H), 10.35 (d, *J* = 10.8 Hz, 0.2H), 9.50 (s, 0.8H), 9.47 (s, 0.2H), 8.84 (d, *J* = 10.8 Hz, 0.2H), 8.37 (d, *J* = 2.4 Hz, 0.8H), 8.34 (d, *J* = 1.8 Hz, 0.8H), 7.77 (s, 0.2H), 7.74 (dd, *J* = 8.9, 2.5 Hz, 0.8H), 7.60 (d, *J* = 8.9 Hz, 0.8H), 7.53 (d, *J* = 1.5 Hz, 0.4H), 1.45 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 160.6, 153.0, 141.5, 134.8, 128.5, 125.6, 125.0, 115.5, 80.8, 28.4. HRMS calcd for C₁₂H₁₅N₃NaO₅⁺ [M+Na]⁺ 304.0909, found 304.0904.



Compound 35. To a solution of compound **34** (205 mg, 0.73 mmol) in MeOH (10 mL) was added 10% Pd/C (21 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **35** (154 mg, 84% yield) as a white solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.90 (s, 0.3H), 9.88 – 9.81 (m, 0.7H), 8.57 (dd, *J* = 11.1, 1.8 Hz, 0.3H), 8.19 (s, 0.7H), 8.14 (d, *J* = 2.0 Hz, 1H), 7.14 – 6.90 (m, 1.7H), 6.67 – 6.41 (m, 0.7H), 6.44 (t, *J* = 2.2 Hz, 0.3H), 6.35 (dd, *J* = 8.5, 2.4 Hz, 0.3H), 4.96 (s, 2H), 1.43 (s, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 162.8, 159.6, 154.4, 143.0, 142.2, 136.0, 125.7, 120.5, 120.2, 108.2, 107.0, 106.4, 105.4, 79.2, 79.1, 28.8. HRMS calcd for C₁₂H₁₇N₃NaO₃⁺ [M+Na]⁺ 274.1168, found 274.1161.



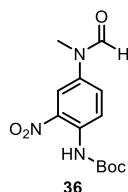
Compound WNO24-Boc. 7-Amino-4-methyl-2H-chromen-2-one (521 mg, 2.2 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.12 mL, 0.73 mmol), DMAP (45 mg, 0.37 mmol), and compound **35** (184 mg, 0.73 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO24-Boc** (68 mg, 18% yield) as a white solid. ¹H NMR (500 MHz, *d*⁶-DMSO) δ 10.28 – 10.08 (m, 1H), 9.75 (d, *J* = 13.8 Hz, 1H), 8.48 (s, 1H), 8.23 (d, *J* = 1.9 Hz, 1H), 8.17 (s, 1H), 8.05 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.35 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.30 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.19 (s, 1H), 6.21 (d, *J* = 1.3 Hz, 1H), 2.39 (d, *J* = 1.2 Hz, 3H), 1.43 (s, 9H). ¹³C NMR (126 MHz, *d*⁶-DMSO) δ 162.9, 160.6, 159.9, 154.8, 154.5, 154.4, 153.7, 152.8, 143.9, 136.0, 126.7, 126.5, 115.1, 114.7, 114.3, 113.5, 112.0, 104.6, 79.7, 28.6, 18.5. HRMS calcd for C₂₃H₂₃N₄O₆⁻ [M-H]⁻ 451.1618, found 451.1626.



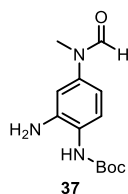
Compound WNO24. To a solution of compound **WNO24-Boc** (68 mg, 0.15 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.6 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO24** (27 mg, 33% yield) as a white solid. ¹H NMR (500 MHz, *d*⁶-DMSO) δ 10.24 (s, 1H), 9.76 (d, *J* = 10.5 Hz, 1H), 8.84 (s, 1H), 8.24 (d, *J* = 1.9 Hz, 1H), 7.73 – 7.62 (m, 3H), 7.42 – 7.30 (m, 2H), 7.09 (dd, *J* = 20.6, 8.6 Hz, 1H),

6.22 (d, $J = 1.4$ Hz, 1H), 2.39 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 163.6, 163.1, 160.7, 160.2, 159.1, 158.8, 154.4, 153.9, 153.5, 143.8, 130.6, 126.7, 126.5, 122.8, 118.0, 117.0, 116.3, 115.1, 114.5, 112.1, 108.0, 105.8, 105.1, 102.3, 99.0, 18.5. HRMS calcd for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{NaO}_4^+$ $[\text{M}+\text{Na}]^+$ 375.1069, found 375.1061.

Synthesis of WNO25

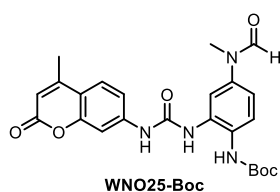


Compound 36. To a Schlenk flask was added $\text{Pd}(\text{OAc})_2$ (224 mg, 1 mmol), Xantphos (867 mg, 1.5 mmol), Cs_2CO_3 (2.43 g, 7.5 mmol), and anhydrous 1,4-dioxane (15 mL). The mixture was stirred at room temperature for 0.5 h. To this mixture was added a solution of compound **17** (1.58 g, 5 mmol) in anhydrous 1,4-dioxane (15 mL) and *N*-methylformamide (354 mg, 6 mmol). The resulting mixture was heated to 100 °C for 24 h. After cooled to room temperature, the reaction mixture was filtered with celite and washed with EA three times. The combined organic layers were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:10) to afford compound **36** (369 mg, 25% yield) as a yellow solid. ^1H NMR (500 MHz, CDCl_3) δ 9.60 (s, 1H), 8.66 (d, $J = 9.2$ Hz, 0.88H), 8.60 (d, $J = 9.3$ Hz, 0.12H), 8.49 (s, 0.88H), 8.38 (s, 0.12H), 8.30 (d, $J = 2.7$ Hz, 0.12H), 8.01 (d, $J = 2.7$ Hz, 0.88H), 7.82 (dd, $J = 9.3, 2.7$ Hz, 0.12H), 7.46 (dd, $J = 9.2, 2.7$ Hz, 0.88H), 3.39 (s, 0.36H), 3.33 (s, 2.64H), 1.56 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 161.7, 152.1, 136.1, 136.0, 134.3, 129.5, 122.3, 119.0, 82.4, 32.1, 28.2. HRMS calcd for $\text{C}_{13}\text{H}_{17}\text{N}_3\text{NaO}_5^+$ $[\text{M}+\text{Na}]^+$ 318.1066, found 318.1061.

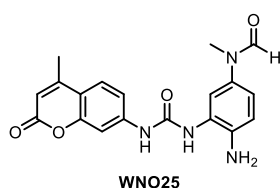


Compound 37. To a solution of compound **36** (369 mg, 0.73 mmol) in MeOH (10 mL) was added 10% Pd/C (37 mg, 10% wt). The reaction mixture was stirred at room temperature under a H_2 atmosphere

overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **37** (266 mg, 80% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 8.40 (s, 1H), 7.27 – 7.24 (m, 1H), 6.57 (d, J = 8.1 Hz, 2H), 6.19 (s, 1H), 3.92 (s, 2H), 3.25 (s, 3H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 162.4, 154.0, 141.6, 140.5, 126.3, 122.8, 113.4, 111.3, 81.1, 77.5, 32.3, 28.4. HRMS calcd for $\text{C}_{13}\text{H}_{19}\text{N}_3\text{NaO}_3^+ [\text{M}+\text{Na}]^+$ 288.1324, found 288.1318.



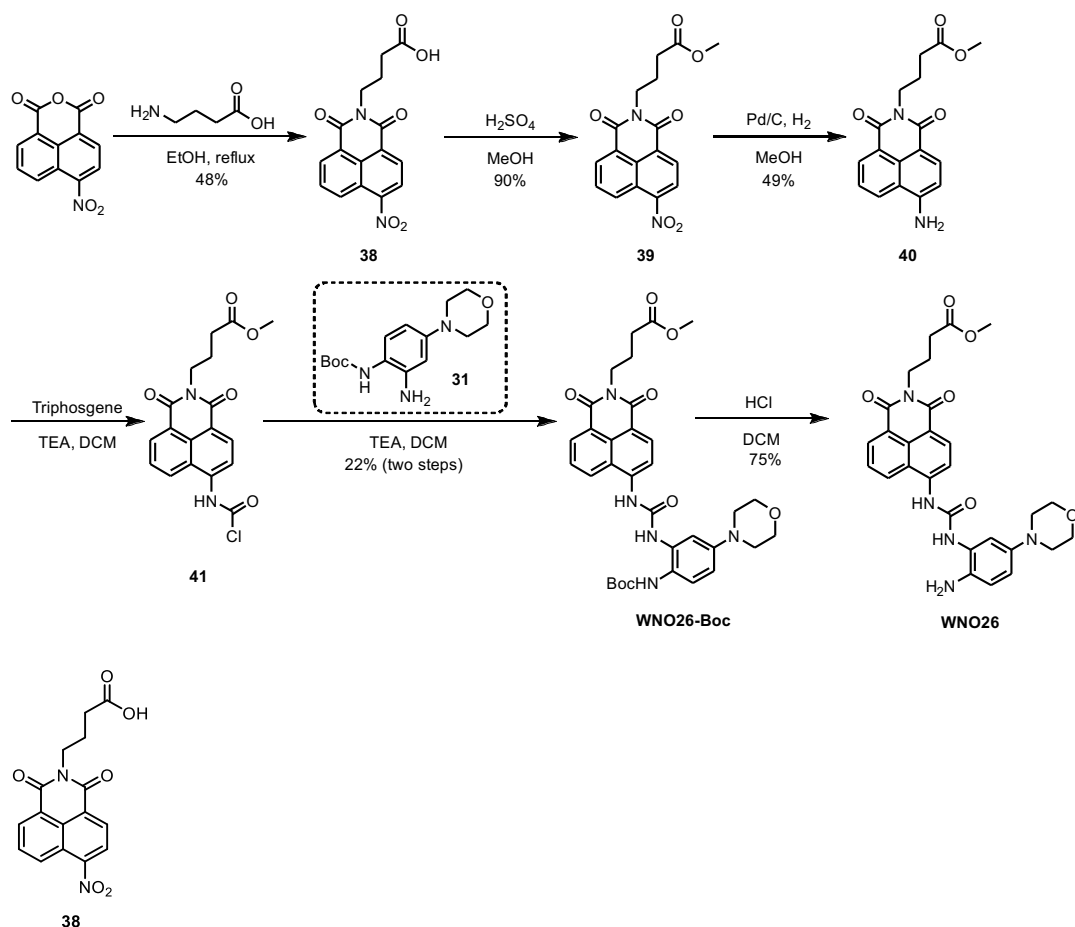
Compound WNO25-Boc. 7-Amino-4-methyl-2H-chromen-2-one (711 mg, 3 mmol) was transformed into the corresponding coumarin-carbonyl chloride **B** according to the general procedure. The crude product was dissolved in anhydrous DCM (10 mL). To this solution was added DIPEA (0.17 mL, 1 mmol), DMAP (61 mg, 0.5 mmol), and compound **37** (266 mg, 1 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO25-Boc** (277 mg, 59% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.75 (s, 1H), 8.63 (s, 1H), 8.53 (s, 1H), 8.33 (d, J = 20.7 Hz, 1H), 7.87 (d, J = 2.7 Hz, 1H), 7.68 – 7.58 (m, 2H), 7.37 (d, J = 8.5 Hz, 1H), 7.30 (dd, J = 8.7, 2.3 Hz, 1H), 7.02 (dd, J = 8.6, 2.7 Hz, 1H), 6.17 (d, J = 2.7 Hz, 1H), 3.45 (s, 0.5H), 3.23 (s, 2.5H), 2.37 (d, J = 2.5 Hz, 3H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 162.4, 160.6, 154.5, 154.3, 153.5, 152.9, 152.8, 143.7, 139.6, 134.2, 127.0, 126.8, 126.3, 116.7, 114.6, 114.3, 112.0, 104.7, 79.9, 31.8, 28.6, 18.4. HRMS calcd for $\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}_6^- [\text{M}-\text{H}]^-$ 465.1774, found 465.1780.



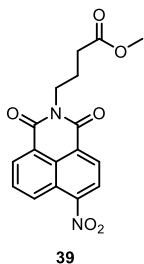
Compound WNO25. To a solution of compound **WNO25-Boc** (277 mg, 0.59 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA; 0.6 mL). The reaction mixture was stirred at room temperature for 4 h. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with PE and DCM to afford compound **WNO25** (182 mg, 63% yield) as a white solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 9.66 (s, 1H), 8.40 (d, $J = 19.4$ Hz, 2H), 7.76 – 7.64 (m, 2H), 7.45 – 7.34 (m, 2H), 6.99 (d, $J = 2.2$ Hz, 2H), 6.23 (d, $J = 1.4$ Hz, 1H), 3.76 – 3.75 (m, 2H), 3.17 (s, 3H), 2.41 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 162.4, 160.7, 154.5, 153.8, 153.3, 144.0, 135.4, 135.1, 127.4, 126.5, 119.8, 119.1, 119.0, 114.8, 114.3, 112.0, 104.8, 32.2, 18.5. HRMS calcd for $\text{C}_{19}\text{H}_{19}\text{N}_4\text{O}_4^+$ $[\text{M}+\text{H}]^+$ 367.1406, found 367.1405.

Synthesis of probes WNO26

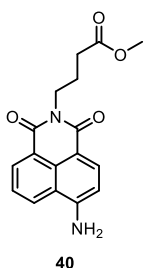
Scheme S4. Synthetic scheme for nitric oxide fluorescent probe **WNO26**.



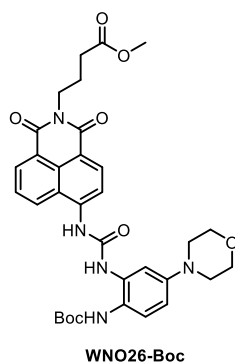
Compound 38. To a solution of 4-nitro-1,8-naphthalic anhydride (5 g, 20.57 mmol) in ethanol (100 mL) was added 4-aminobutanoic acid (2.33 g, 22.63 mmol). The reaction mixture was heated to reflux for 8 h. After cooled to room temperature, the mixture was poured into iced water. The resulting precipitates were filtered and washed with water. The residue was recrystallized with ethanol to afford compound **38** (3.23 g, 48% yield) as a yellow solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 12.05 (s, 1H), 9.43 (d, $J = 2.3$ Hz, 1H), 8.89 (d, $J = 2.3$ Hz, 1H), 8.74 (d, $J = 8.3$ Hz, 1H), 8.64 (d, $J = 7.3$ Hz, 1H), 8.04 (t, $J = 7.8$ Hz, 1H), 4.10 (t, $J = 6.9$ Hz, 2H), 2.35 (t, $J = 7.3$ Hz, 2H), 2.01 – 1.84 (m, 2H).



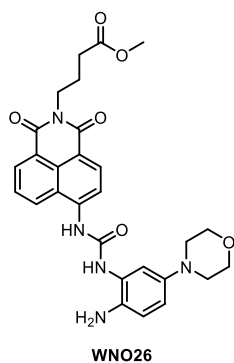
Compound 39. To a solution of compound **38** (3.23 g, 9.85 mmol) in methanol (60 mL) was added concentrated H₂SO₄ (2.3 mL) dropwise. The reaction mixture was heated to reflux overnight. After cooled to room temperature, the mixture was poured into iced water. The resulting precipitates were filtered and washed with water. The residue was recrystallized with ethanol to afford compound **39** (3.03 g, 90% yield) as a yellow solid. ¹H NMR (500 MHz, *d*⁶-DMSO) δ 8.73 – 8.68 (m, 1H), 8.64 – 8.61 (m, 1H), 8.60 (d, *J* = 8.0 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.09 (dd, *J* = 8.7, 7.3 Hz, 1H), 4.09 (t, *J* = 6.9 Hz, 2H), 3.51 (s, 3H), 2.42 (t, *J* = 7.3 Hz, 2H), 1.98 – 1.89 (m, 2H).



Compound 40. To a solution of compound **39** (1.2 g, 2.22 mmol) in MeOH (25 mL) and THF (25 mL) was added 10% Pd/C (120 mg, 10% wt). The reaction mixture was stirred at room temperature under a H₂ atmosphere overnight. The mixture was filtered with celite, washed with MeOH, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:4) to afford compound **40** (358 mg, 49% yield) as a yellow solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 8.62 (dd, *J* = 8.5, 1.2 Hz, 1H), 8.44 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.46 (s, 2H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.06 (t, *J* = 6.9 Hz, 2H), 3.52 (s, 3H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.97 – 1.84 (m, 2H).



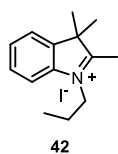
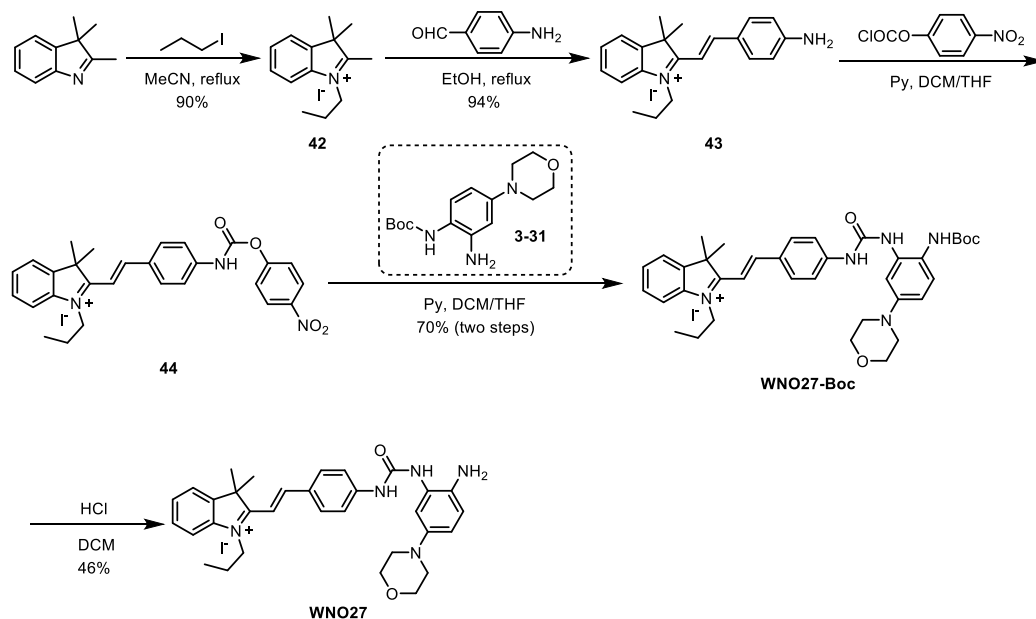
Compound WNO26-Boc. To a solution of compound **40** (60 mg, 0.19 mmol) in anhydrous CH₂Cl₂ (5 mL) was added triethylamine (0.03 mL, 0.19 mmol) and a solution of triphosgene (28 mg, 0.095 mmol) in anhydrous CH₂Cl₂ (5 mL). The reaction mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was vacuumed on an oil pump for 2 h to provide the corresponding carbonyl chloride **41**. The crude product was redissolved in anhydrous CH₂Cl₂ (10 mL). To this solution was added triethylamine (0.05 mL, 0.38 mmol) and compound **31** (67 mg, 0.23 mmol) successively. The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H₂O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EA/PE = 1:2) to afford compound **WNO26-Boc** (27 mg, 22% yield) as a yellow solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 9.91 (s, 1H), 8.71 (d, *J* = 8.6 Hz, 2H), 8.57 (dd, *J* = 7.9, 4.9 Hz, 2H), 8.48 (d, *J* = 8.4 Hz, 1H), 7.96 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.60 (d, *J* = 4.9 Hz, 1H), 7.13 (s, 1H), 6.69 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.11 (t, *J* = 6.8 Hz, 2H), 3.77 (dd, *J* = 6.1, 3.5 Hz, 4H), 3.53 (s, 3H), 3.10 (dd, *J* = 5.8, 3.6 Hz, 4H), 2.42 (t, *J* = 7.3 Hz, 2H), 2.02 – 1.89 (m, 2H), 1.58 – 1.38 (m, 9H). ¹³C NMR (101 MHz, *d*⁶-DMSO) δ 173.4, 164.2, 163.6, 161.7, 160.6, 154.7, 152.9, 149.4, 142.2, 132.9, 131.3, 130.5, 129.1, 128.5, 127.4, 126.7, 123.1, 123.0, 121.2, 115.7, 79.4, 66.6, 51.7, 49.4, 31.5, 28.6, 23.5. HRMS calcd for C₃₃H₃₇N₅NaO₈⁺ [M+Na]⁺ 654.2540, found 654.2536.



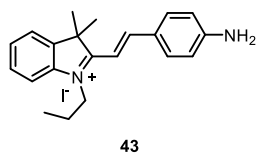
Compound WNO26. To a solution of compound **WNO26-Boc** (20 mg, 0.03 mmol) in DCM (5 mL) was added the HCl solution in 1,4-dioxane (4 M; 0.03 mL, 0.12 mmol). The reaction mixture was stirred at room temperature overnight. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with methanol and diethyl ether to afford compound **WNO26** (12 mg, 75% yield) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 9.75 (s, 1H), 8.79 (d, $J = 1.1$ Hz, 1H), 8.78 – 8.69 (m, 1H), 8.57 (d, $J = 8.5$ Hz, 1H), 8.50 (dd, $J = 7.3, 0.9$ Hz, 1H), 8.41 (d, $J = 8.5$ Hz, 1H), 7.88 (dd, $J = 8.6, 7.3$ Hz, 1H), 7.27 (d, $J = 2.7$ Hz, 1H), 6.72 (d, $J = 8.6$ Hz, 1H), 6.57 (dd, $J = 8.6, 2.7$ Hz, 1H), 4.66 (s, 1H), 4.07 (t, $J = 6.9$ Hz, 2H), 3.80 – 3.66 (m, 4H), 3.51 (s, 3H), 2.99 – 2.88 (m, 4H), 2.45 – 2.36 (m, 2H), 1.97 – 1.87 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 178.2, 169.0, 168.4, 157.7, 148.5, 147.4, 138.9, 137.7, 136.0, 134.9, 133.8, 133.3, 131.2, 130.6, 127.7, 127.4, 122.1, 120.0, 119.8, 118.3, 116.9, 71.6, 56.5, 55.6, 36.2, 28.2. HRMS calcd for $\text{C}_{28}\text{H}_{30}\text{N}_5\text{O}_6^+$ $[\text{M}+\text{H}]^+$ 532.2196, found 532.2193.

Synthesis of probes WNO27

Scheme S5. Synthetic scheme for nitric oxide fluorescent probe **WNO27**.

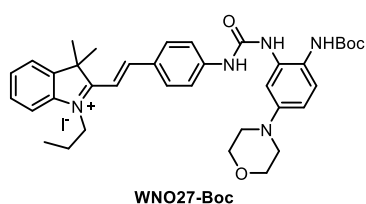


Compound 42. To a solution of 2,3,3-trimethylindolenine (5 g, 31.4 mmol) in acetonitrile (200 mL) was added 1-iodopropane (15.2 mL, 157 mmol). The reaction mixture was heated to reflux for 15 h. After cooled to room temperature, the precipitates were filtered and washed with ether. The residue was recrystallized with acetone to afford compound **42** (9.33 g, 90% yield) as a purple solid. ¹H NMR (400 MHz, *d*⁶-DMSO) δ 8.09 – 7.98 (m, 1H), 7.92 – 7.82 (m, 1H), 7.70 – 7.60 (m, 2H), 4.55 – 4.41 (m, 2H), 2.88 (s, 3H), 1.99 – 1.83 (m, 2H), 1.56 (s, 6H), 1.01 (t, *J* = 7.4 Hz, 3H).

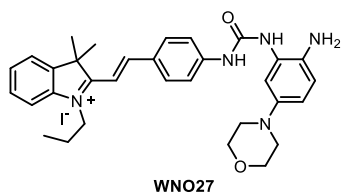


Compound 43. To a solution of compound **42** (1.09 g, 3.32 mmol) in ethanol (50 mL) was added 4-aminobenzaldehyde (481 mg, 3.98 mmol). The reaction mixture was heated to reflux overnight. After

cooled to room temperature, the mixture was poured into diethyl ether (200 mL). The resulting precipitates were filtered and washed with ether. The residue was dried to afford compound **43** (1.35 g, 94% yield) as a yellow solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 8.25 (d, J = 15.5 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.72 (dd, J = 16.5, 7.6 Hz, 2H), 7.54 – 7.39 (m, 2H), 7.20 (d, J = 15.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 2H), 5.44 (s, 2H), 4.45 (t, J = 7.2 Hz, 2H), 1.77 (q, J = 7.2 Hz, 2H), 1.70 (s, 6H), 0.94 (t, J = 7.3 Hz, 3H).



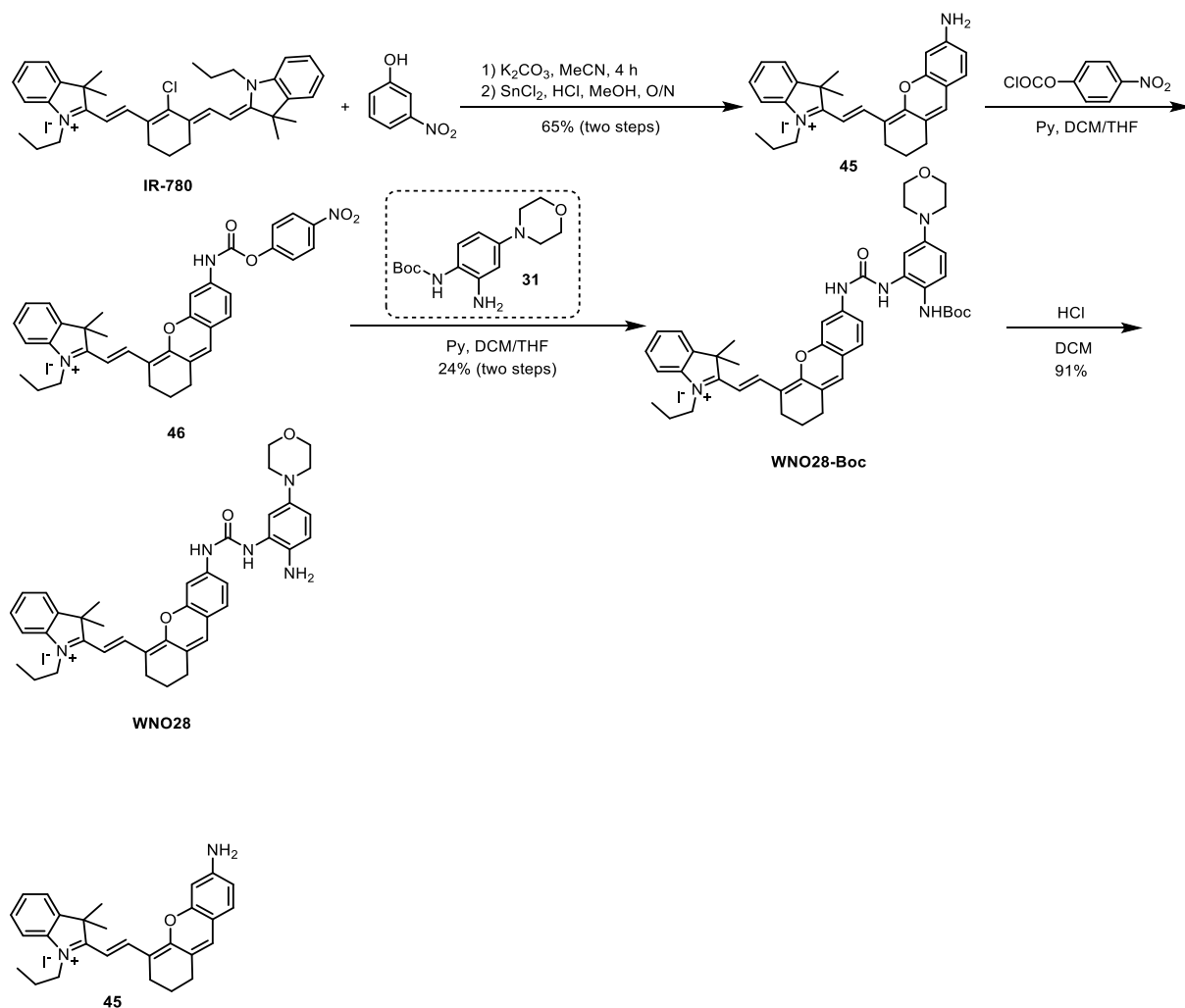
Compound WNO27-Boc. To a solution of compound **43** (96 mg, 0.31 mmol) in anhydrous CH_2Cl_2 (8 mL) was added pyridine (40 μL , 0.49 mmol) and a solution of 4-nitrophenyl chloroformate (200 mg, 0.99 mmol) in anhydrous THF (2 mL) at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 3 h. To this mixture was further added a solution of compound **31** (363 mg, 1.24 mmol) in anhydrous THF (6 mL) and pyridine (2 mL, 24.7 mmol) under the protection of N_2 . The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/MeOH = 10:1) to afford compound **WNO27-Boc** (164 mg, 70% yield) as a yellow solid. ^1H NMR (400 MHz, d^6 -DMSO) δ 10.03 (s, 1H), 8.46 (d, J = 16.0 Hz, 1H), 8.36 (s, 1H), 8.33 – 8.19 (m, 3H), 7.97 – 7.87 (m, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.66 – 7.59 (m, 2H), 7.55 (d, J = 13.5 Hz, 1H), 7.16 – 7.07 (m, 1H), 6.67 (dd, J = 9.0, 2.7 Hz, 1H), 4.74 – 4.59 (m, 2H), 3.84 – 3.71 (m, 4H), 3.16 – 3.02 (m, 4H), 1.96 – 1.87 (m, 2H), 1.82 (s, 6H), 1.47 (s, 9H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, d^6 -DMSO) δ 182.0, 154.8, 154.6, 152.8, 149.5, 146.1, 144.1, 141.4, 134.5, 133.2, 130.2, 129.6, 129.5, 128.3, 127.4, 123.6, 120.9, 118.2, 115.5, 110.1, 79.4, 66.6, 52.5, 49.3, 47.8, 28.6, 26.5, 22.2, 11.3. HRMS calcd for $\text{C}_{37}\text{H}_{46}\text{N}_5\text{O}_4^+$ $[\text{M}]^+$ 624.3544, found 624.3537.



Compound WNO27. To a solution of compound **WNO27-Boc** (54 mg, 0.07 mmol) in DCM (5 mL) was added the HCl solution in 1,4-dioxane (4 M; 0.07 mL, 0.28 mmol). The reaction mixture was stirred at room temperature overnight. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with methanol and diethyl ether to afford compound **WNO27** (21 mg, 46% yield) as a yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 8.44 (d, $J = 16.1$ Hz, 2H), 8.15 – 8.04 (m, 3H), 7.88 – 7.75 (m, 5H), 7.67 – 7.62 (m, 2H), 7.56 (d, $J = 16.1$ Hz, 2H), 7.32 – 7.22 (m, 2H), 7.02 (d, $J = 8.1$ Hz, 2H), 4.64 (t, $J = 7.4$ Hz, 2H), 3.90 – 3.85 (m, 4H), 3.29 – 3.26 (m, 4H), 2.02 (d, $J = 7.1$ Hz, 2H), 1.86 (s, 6H), 1.11 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 182.3, 179.2, 178.1, 166.4, 154.8, 154.0, 145.1, 143.7, 141.0, 132.1, 129.5, 129.3, 128.8, 123.6, 122.8, 118.8, 114.7, 112.0, 109.7, 101.1, 66.1, 52.5, 49.4, 48.4, 25.6, 21.9, 10.0. HRMS calcd for $\text{C}_{32}\text{H}_{38}\text{N}_5\text{O}_2^+ [\text{M}]^+$ 524.3020, found 524.3018.

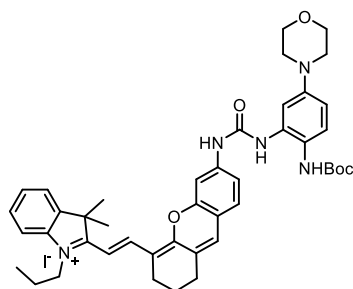
Synthesis of probes WNO28

Scheme S6. Synthetic scheme for nitric oxide fluorescent probe **WNO28**.



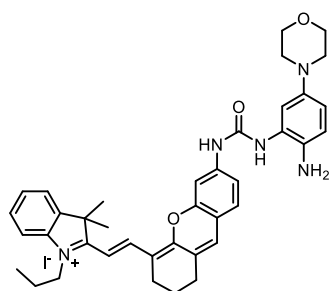
Compound 45. To a solution of 3-nitrophenol (521 mg, 3.75 mmol) in acetonitrile (10 mL) was added K_2CO_3 (519 mg, 3.75 mmol) and **IR-780** (1 g, 1.5 mmol). The reaction mixture was heated to 50 °C for 4 h. After cooled to room temperature, the mixture was concentrated under reduced pressure. The residue was dissolved in DCM (100 mL), washed with water and brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The resulting residue was redissolved in methanol (50 mL). To this solution was added stannous chloride (2.84 g, 15 mmol) and concentrated HCl (3.2 mL). The reaction mixture was heated to 70 °C overnight. After that, the reaction was quenched with saturated $NaHCO_3$ solution, and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The

residue was purified by silica gel column chromatography (DCM/MeOH = 10:1) to afford compound **45** (521 mg, 65% yield) as a purple solid. ^1H NMR (400 MHz, CD_3OD) δ 8.66 (d, $J = 14.3$ Hz, 1H), 7.65 – 7.54 (m, 2H), 7.55 – 7.45 (m, 1H), 7.43 – 7.30 (m, 3H), 6.80 (dd, $J = 8.6, 2.1$ Hz, 1H), 6.74 (d, $J = 2.1$ Hz, 1H), 6.29 (d, $J = 14.3$ Hz, 1H), 4.21 (t, $J = 7.3$ Hz, 2H), 2.81 (t, $J = 6.1$ Hz, 2H), 2.74 (t, $J = 6.2$ Hz, 2H), 2.05 – 1.87 (m, 4H), 1.82 (s, 6H), 1.10 (t, $J = 7.4$ Hz, 3H).



WNO28-Boc

Compound WNO28-Boc. To a solution of compound **45** (80 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (4 mL) was added pyridine (20 μL , 0.25 mmol) and a solution of 4-nitrophenyl chloroformate (90 mg, 0.45 mmol) in anhydrous THF (1 mL) at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 3 h. To this mixture was further added a solution of compound **31** (142 mg, 0.6 mmol) in anhydrous THF (3 mL) and pyridine (1 mL, 12.4 mmol) under the protection of N_2 . The reaction mixture was stirred at room temperature overnight. After completion, the mixture was diluted with H_2O (20 mL) and extracted with DCM three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/MeOH = 10:1) to afford compound **WNO28-Boc** (29 mg, 24% yield) as a purple solid. ^1H NMR (400 MHz, CD_3OD) δ 8.71 (d, $J = 14.9$ Hz, 1H), 8.01 (d, $J = 2.0$ Hz, 1H), 7.85 (d, $J = 2.7$ Hz, 1H), 7.66 (d, $J = 7.2$ Hz, 1H), 7.58 – 7.44 (m, 3H), 7.40 (dd, $J = 8.7, 4.9$ Hz, 2H), 7.27 (s, 1H), 7.11 (dd, $J = 8.4, 2.1$ Hz, 1H), 7.07 – 6.97 (m, 1H), 6.52 (d, $J = 14.9$ Hz, 1H), 4.18 (t, $J = 7.5$ Hz, 2H), 3.88 (t, $J = 4.7$ Hz, 4H), 2.81 – 2.65 (m, 4H), 1.93 (d, $J = 6.1$ Hz, 2H), 1.89 (d, $J = 7.8$ Hz, 2H), 1.85 (s, 6H), 1.54 (s, 9H), 1.04 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 177.8, 161.6, 155.4, 154.0, 153.1, 145.7, 143.5, 142.2, 141.8, 134.0, 133.5, 129.0, 128.2, 127.7, 127.1, 122.5, 117.0, 115.8, 114.4, 113.1, 112.7, 111.6, 109.5, 108.1, 105.1, 104.1, 103.7, 80.3, 65.6, 51.7, 50.7, 46.1, 28.9, 27.4, 23.9, 20.9, 20.3, 10.3. HRMS calcd for $\text{C}_{44}\text{H}_{52}\text{N}_5\text{O}_5^+$ $[\text{M}]^+$ 730.3963, found 730.3966.



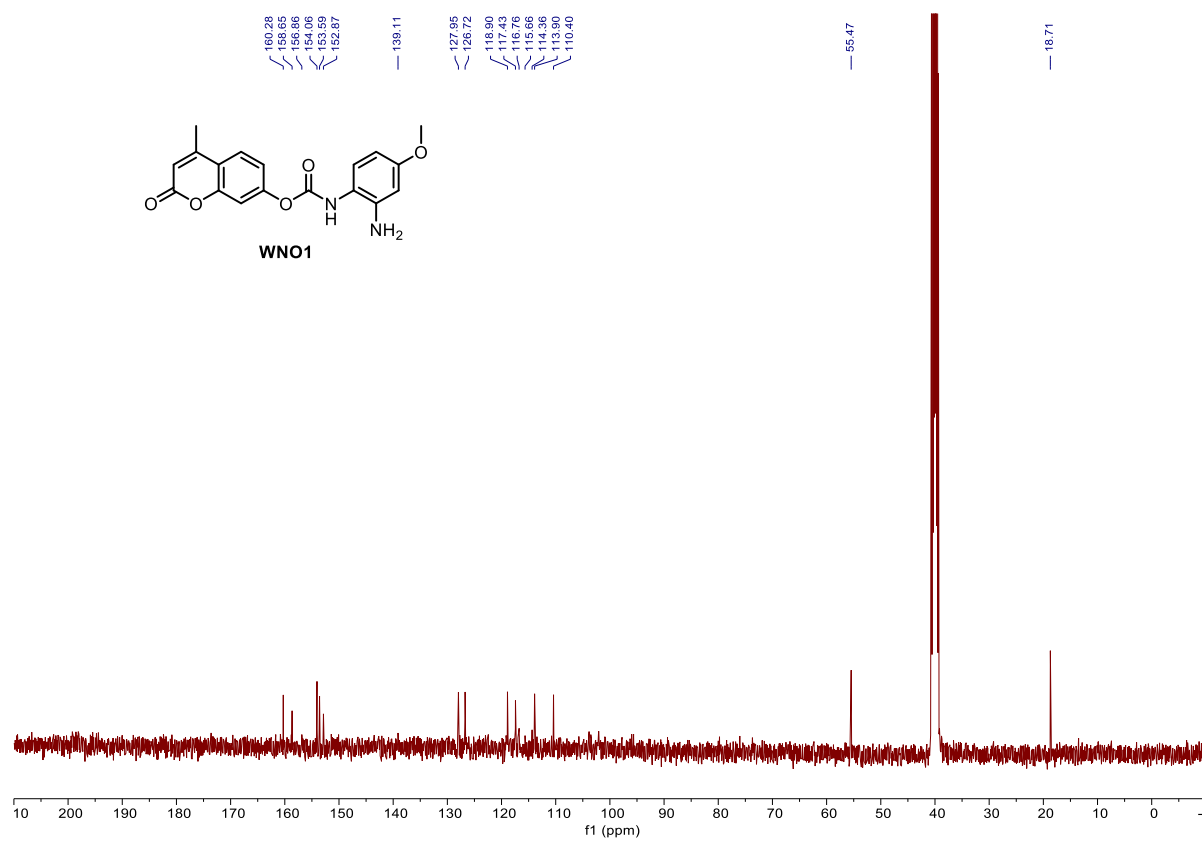
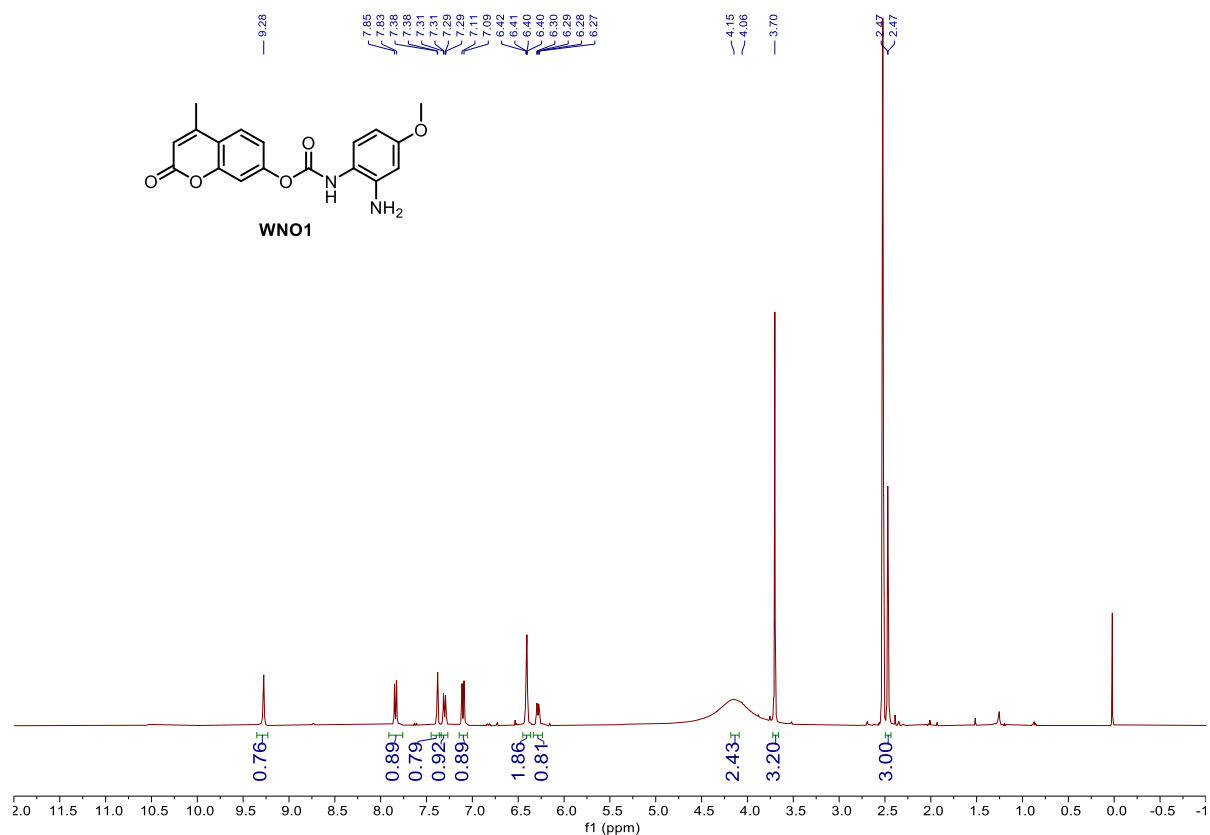
WNO28

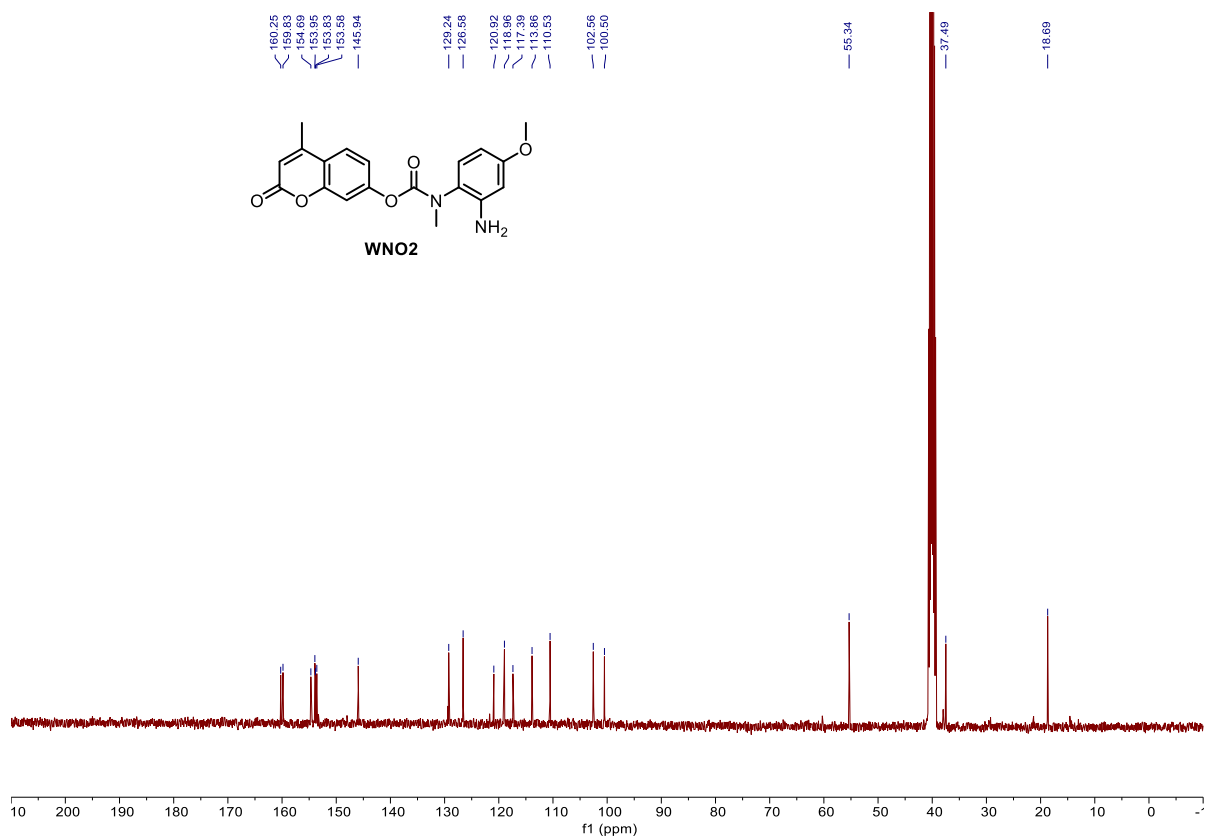
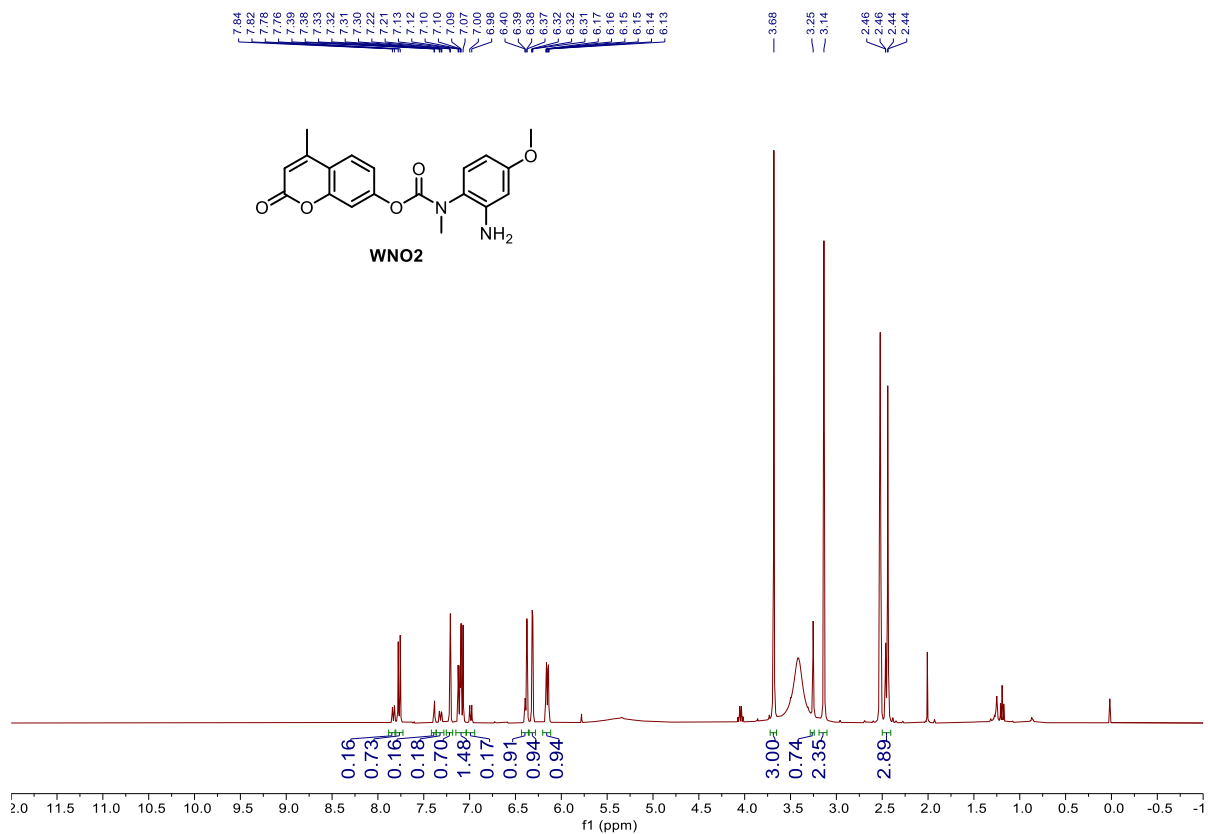
Compound WNO28. To a solution of compound **WNO28-Boc** (100 mg, 0.12 mmol) in DCM (5 mL) was added the HCl solution in 1,4-dioxane (4 M; 0.12 mL, 0.48 mmol). The reaction mixture was stirred at room temperature overnight. After completion, the mixture was concentrated under reduced pressure. The resulting crude product was recrystallized with methanol and diethyl ether to afford compound **WNO28** (83 mg, 91% yield) as a purple solid. ^1H NMR (400 MHz, CD_3OD) δ 8.78 (d, J = 14.9 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 7.68 (d, J = 7.4 Hz, 1H), 7.57 (t, J = 5.0 Hz, 2H), 7.54 – 7.45 (m, 2H), 7.45 – 7.32 (m, 3H), 7.23 (d, J = 2.7 Hz, 1H), 7.14 (dd, J = 8.9, 2.7 Hz, 1H), 6.56 (d, J = 15.0 Hz, 1H), 4.36 (t, J = 7.4 Hz, 2H), 3.95 (t, J = 4.8 Hz, 4H), 3.40 – 3.35 (m, 4H), 2.82 (t, J = 6.0 Hz, 2H), 2.75 (d, J = 6.2 Hz, 2H), 1.97 (q, J = 7.3, 5.7 Hz, 4H), 1.85 (s, 6H), 1.11 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 177.9, 161.5, 153.9, 153.8, 148.5, 145.7, 143.1, 142.1, 141.7, 133.4, 131.7, 128.9, 128.1, 128.0, 127.5, 127.1, 123.7, 122.5, 117.2, 116.2, 114.4, 114.3, 112.9, 112.7, 104.5, 103.7, 65.8, 50.7, 50.0, 46.3, 28.8, 27.1, 23.7, 20.9, 20.3, 10.3. HRMS calcd for $\text{C}_{39}\text{H}_{44}\text{N}_5\text{O}_3^+$ $[\text{M}]^+$ 630.3439, found 630.3439.

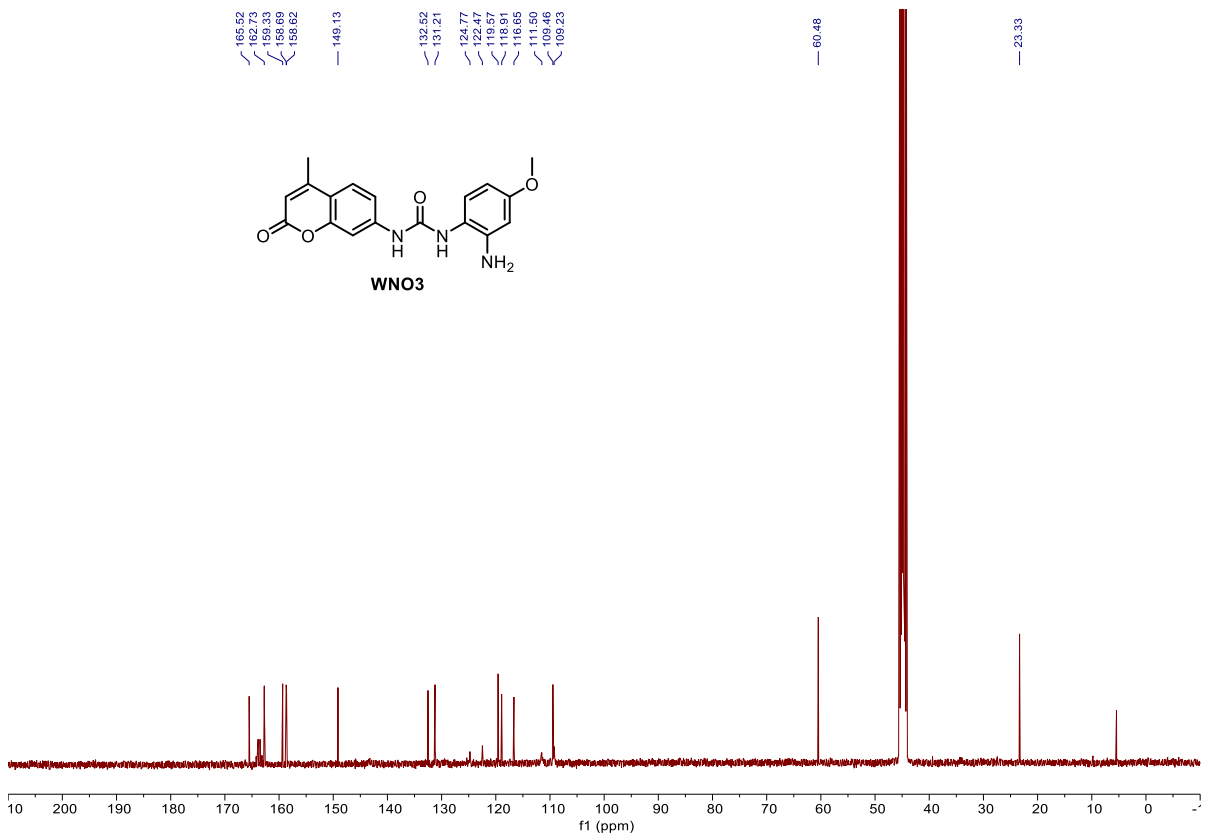
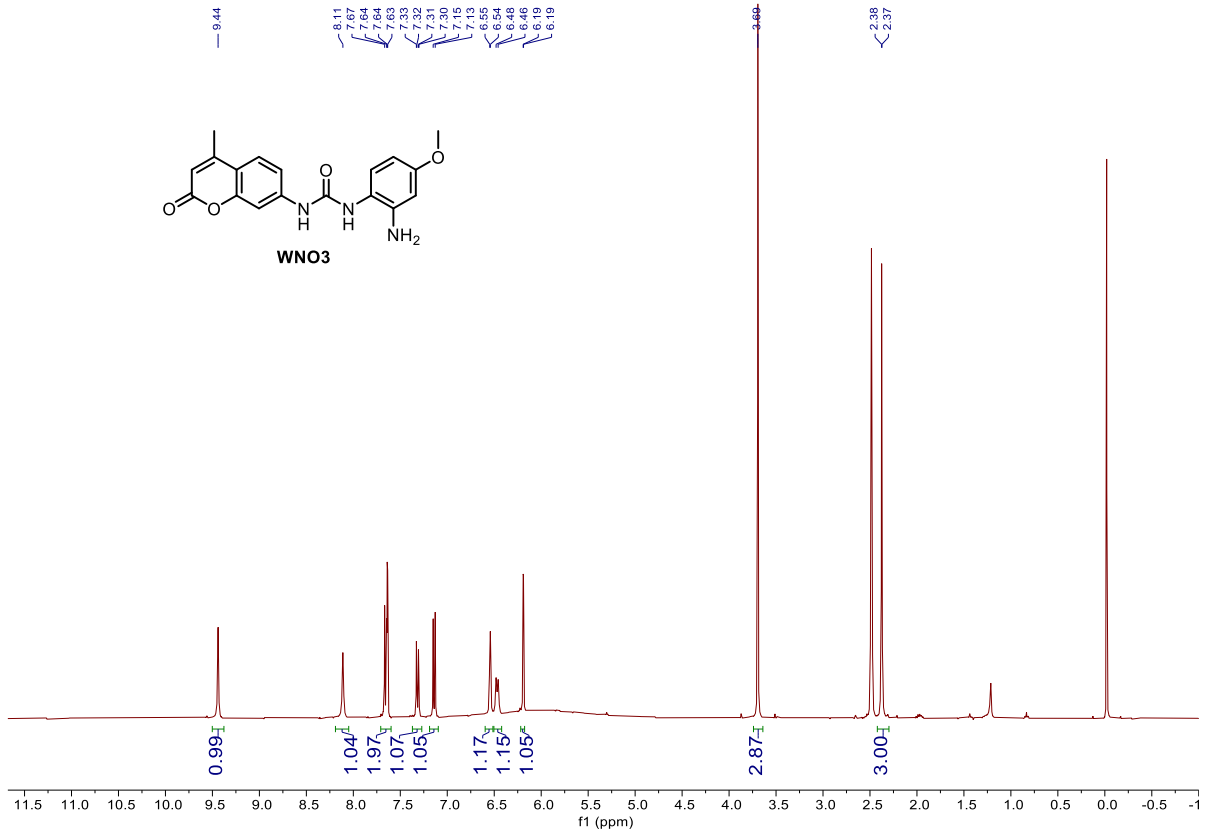
References

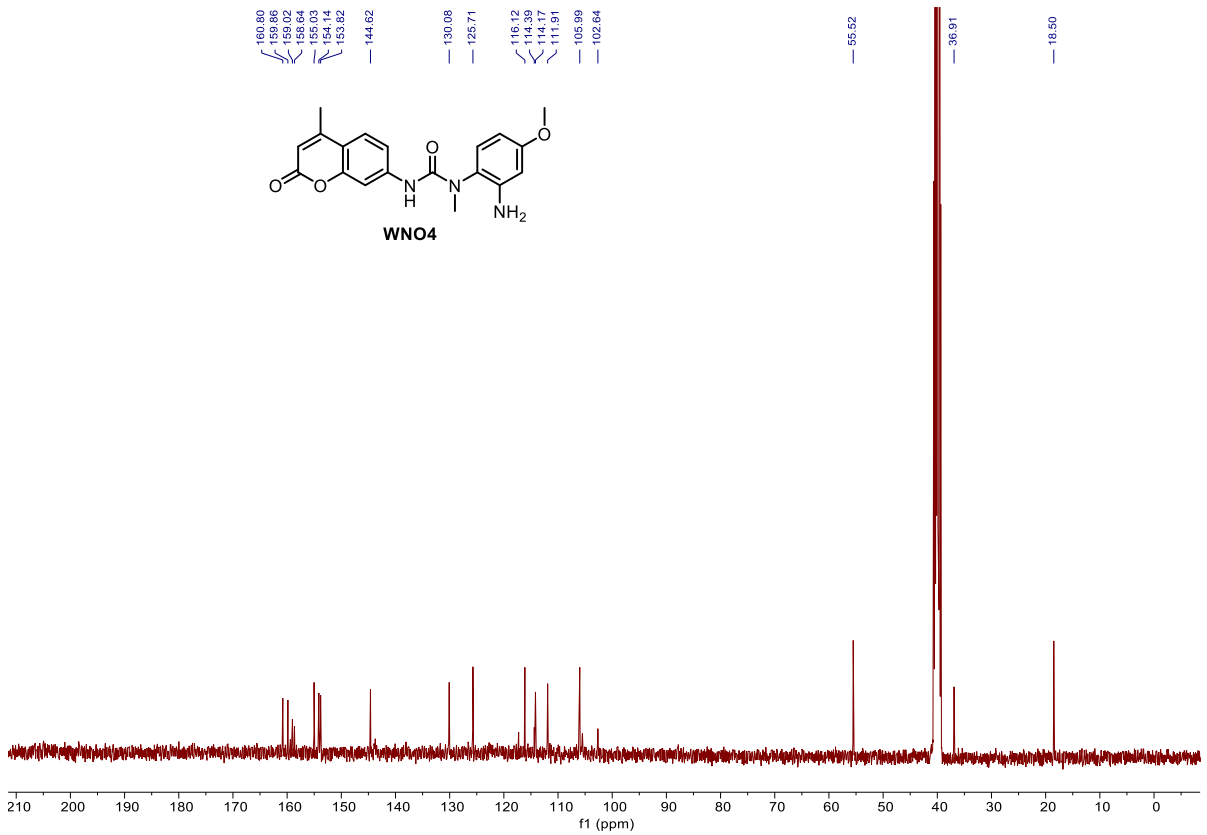
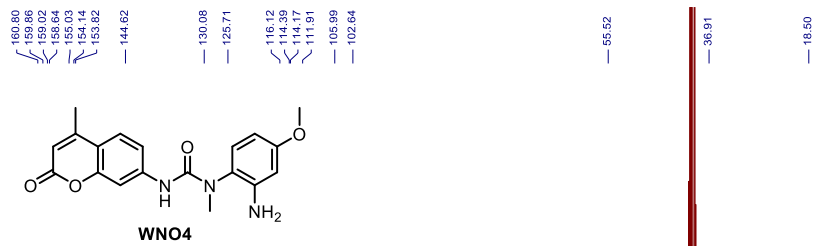
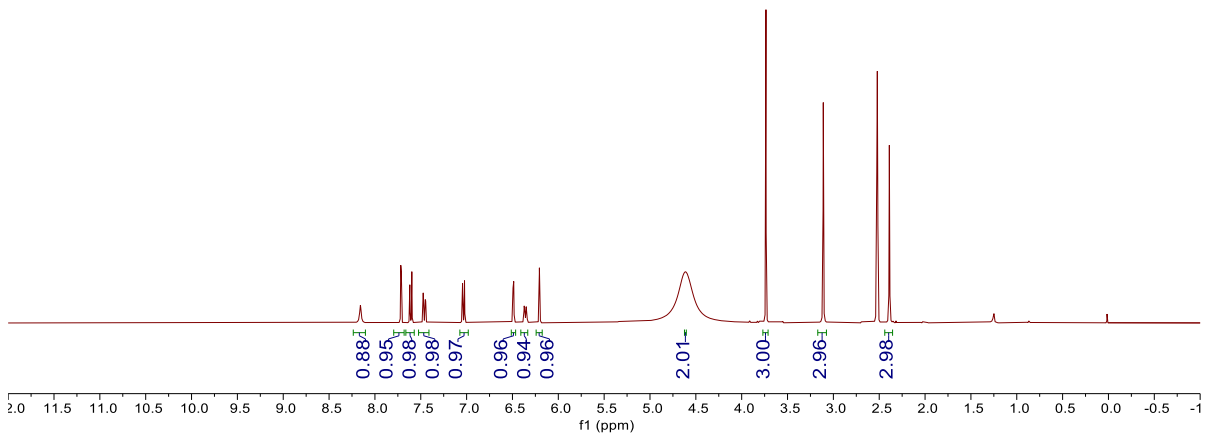
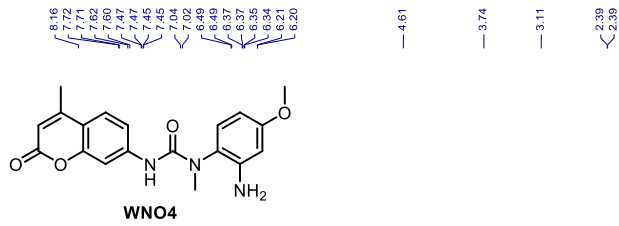
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3. T. Peng, X. Chen, L. Gao, T. Zhang, W. Wang, J. Shen and D. Yang, *Chemical Science*, 2016, **7**, 5407-5413.

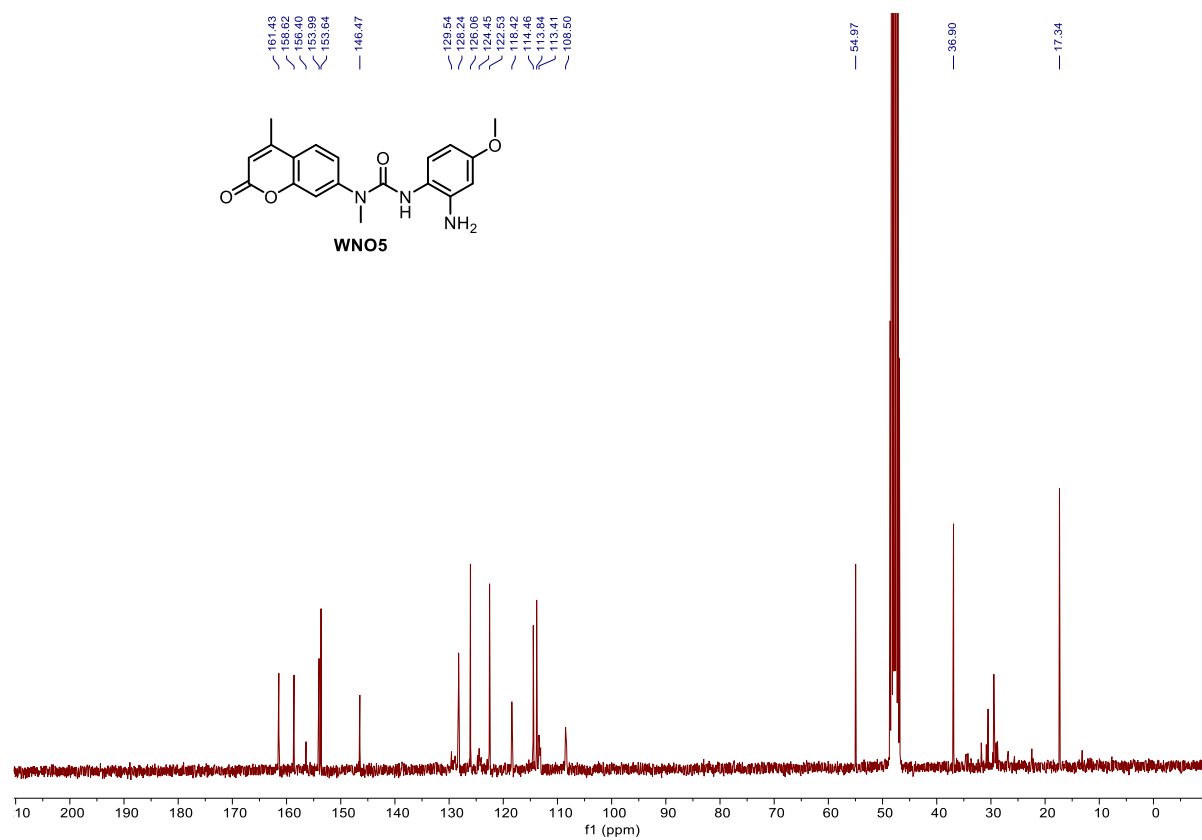
NMR spectra

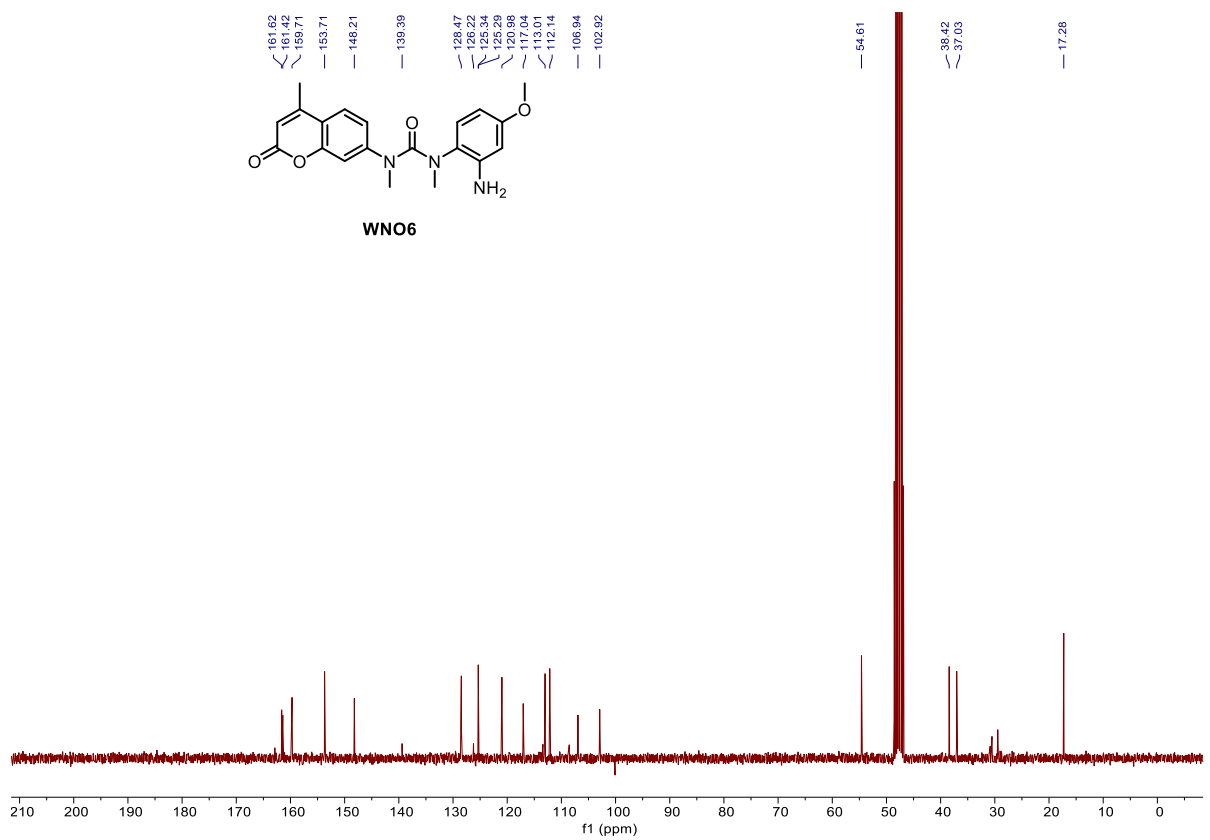
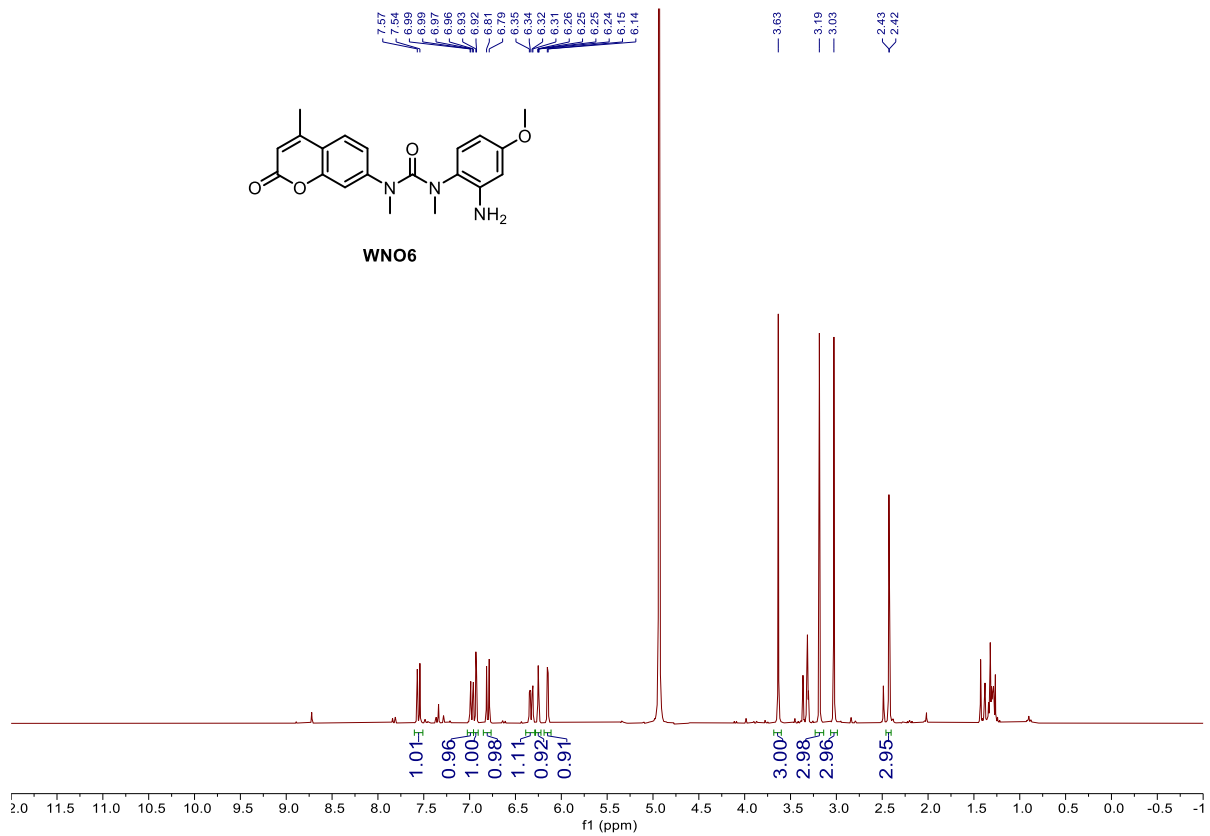


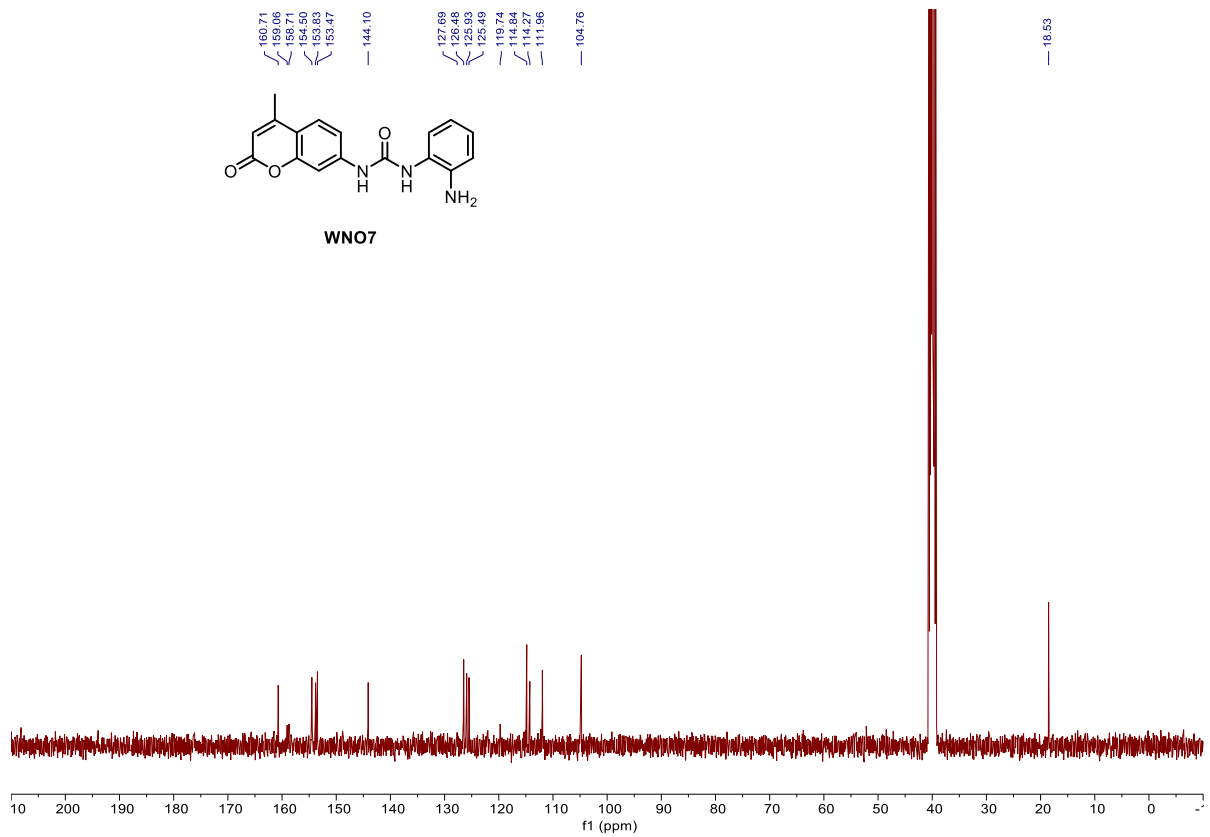
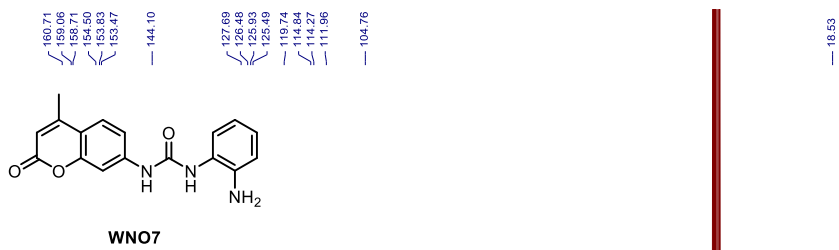
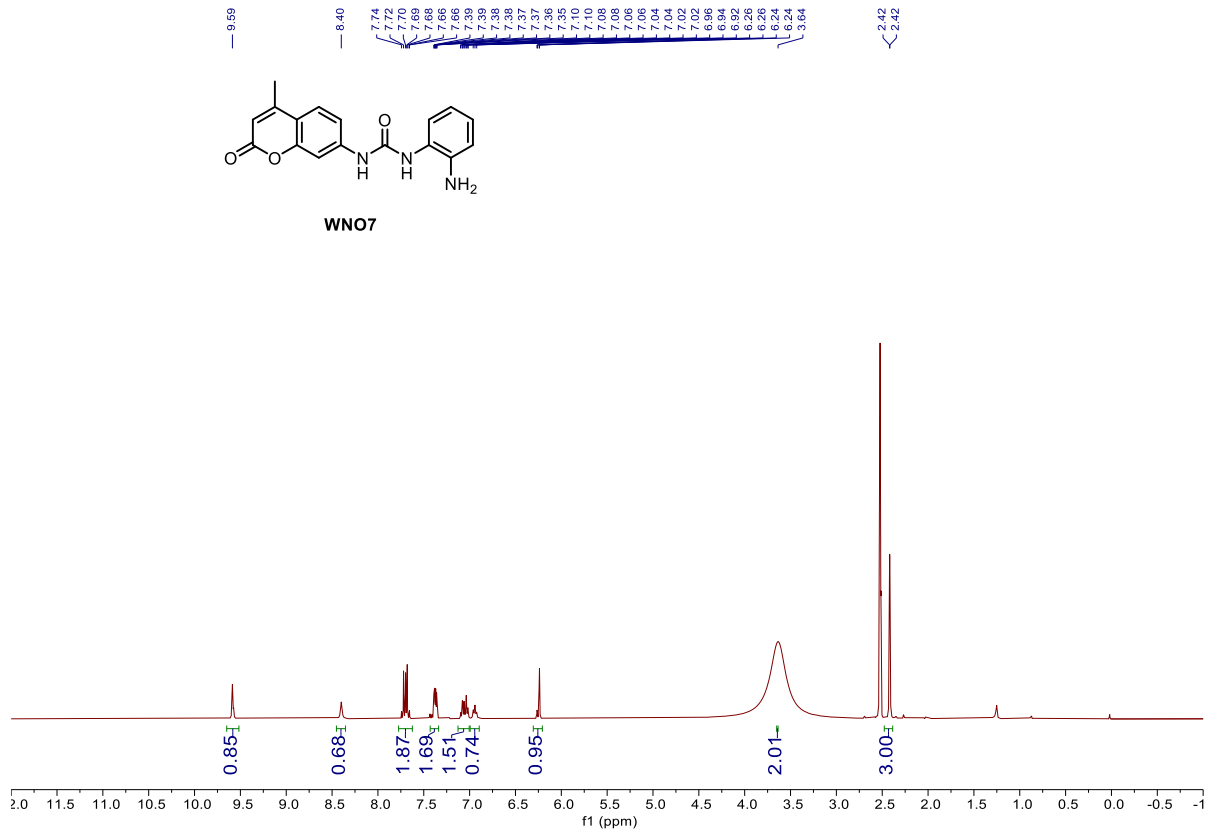
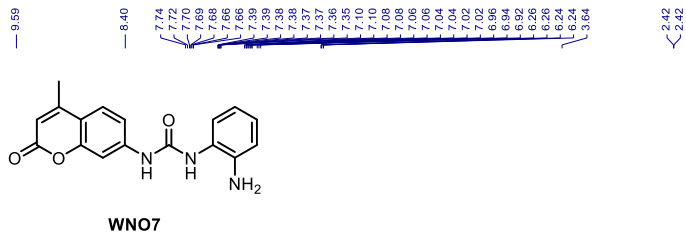


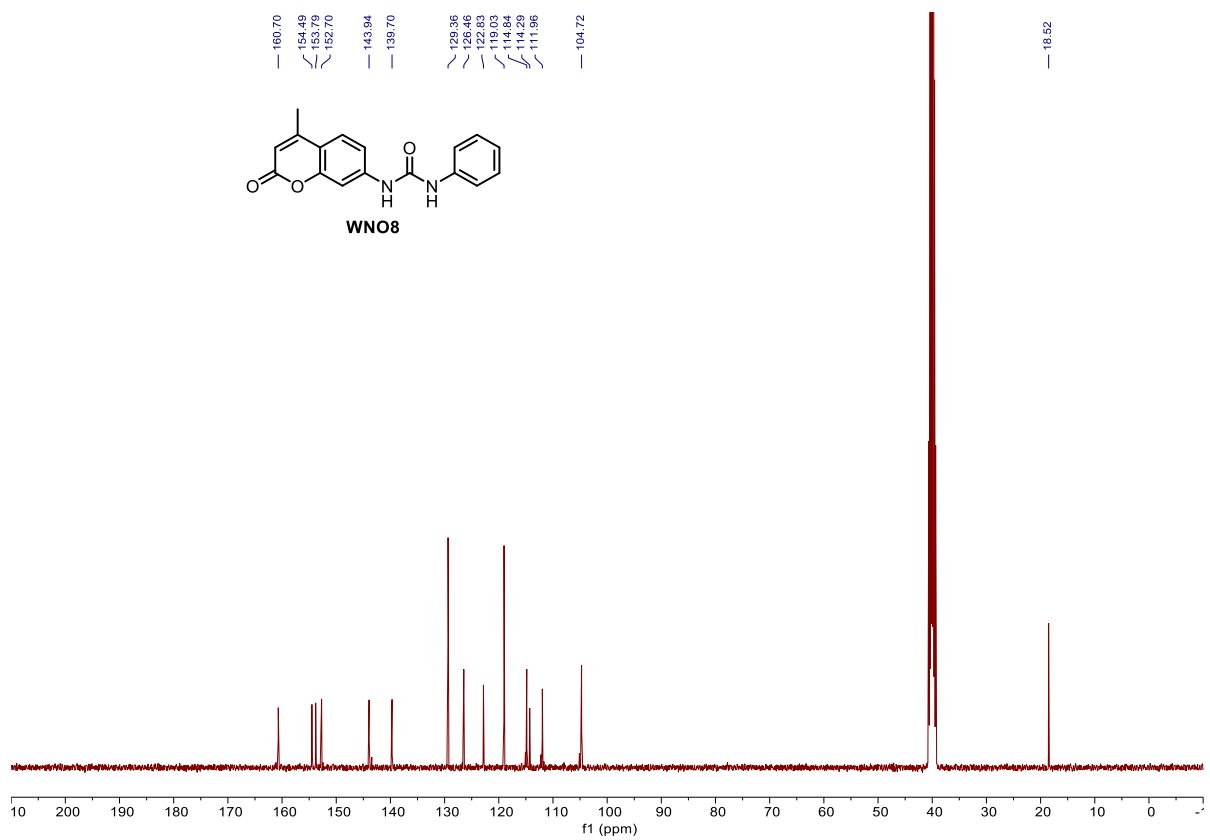
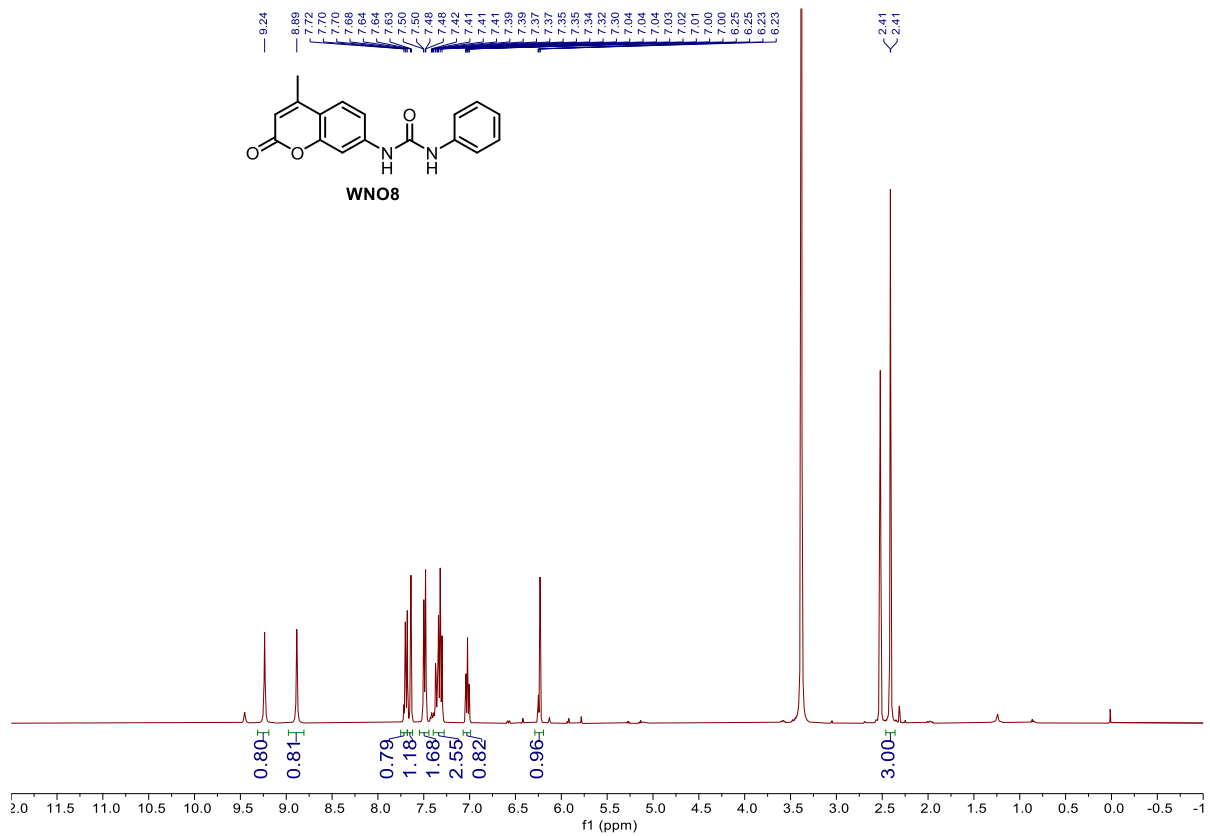


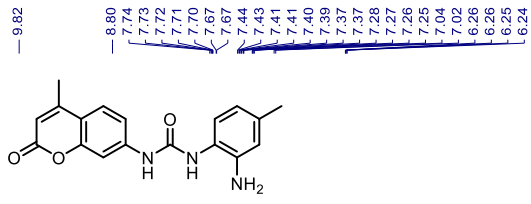




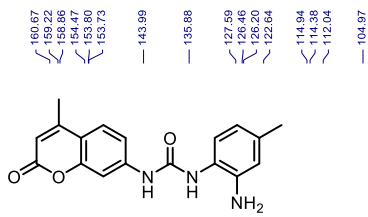
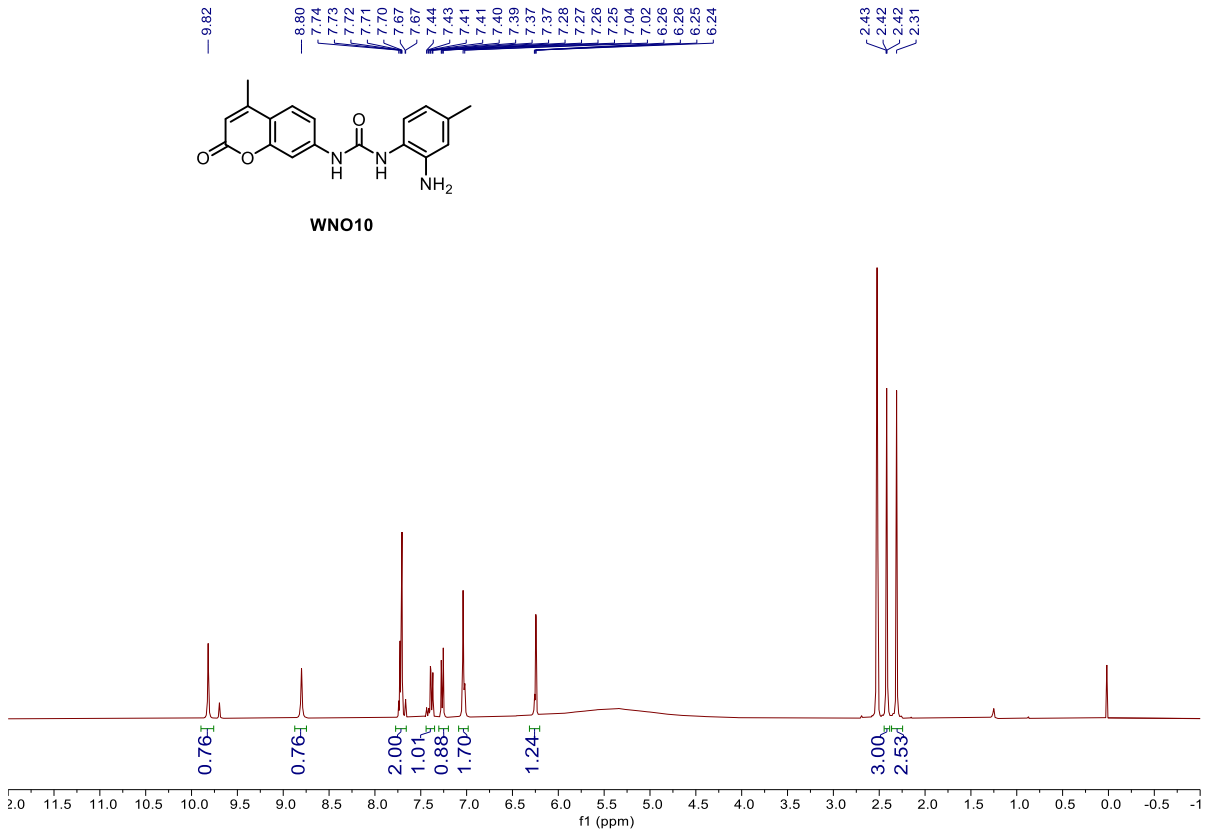




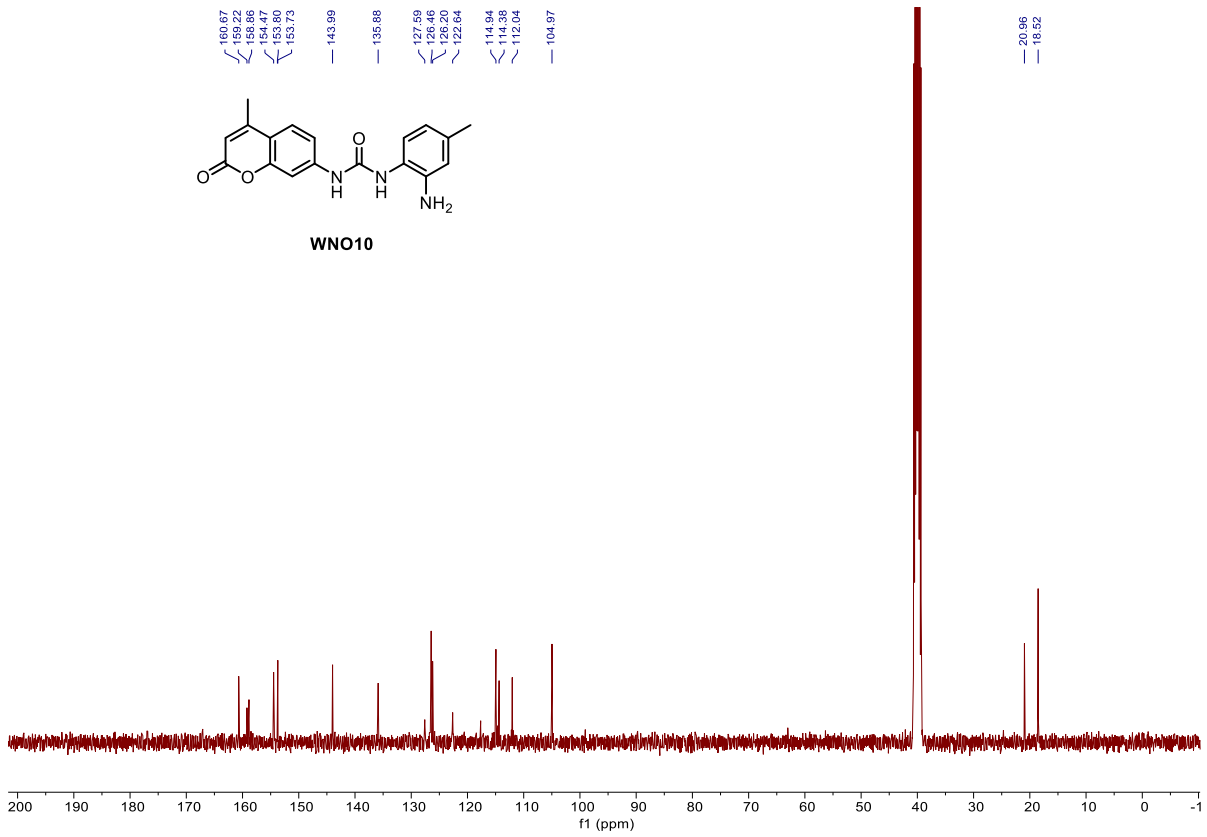


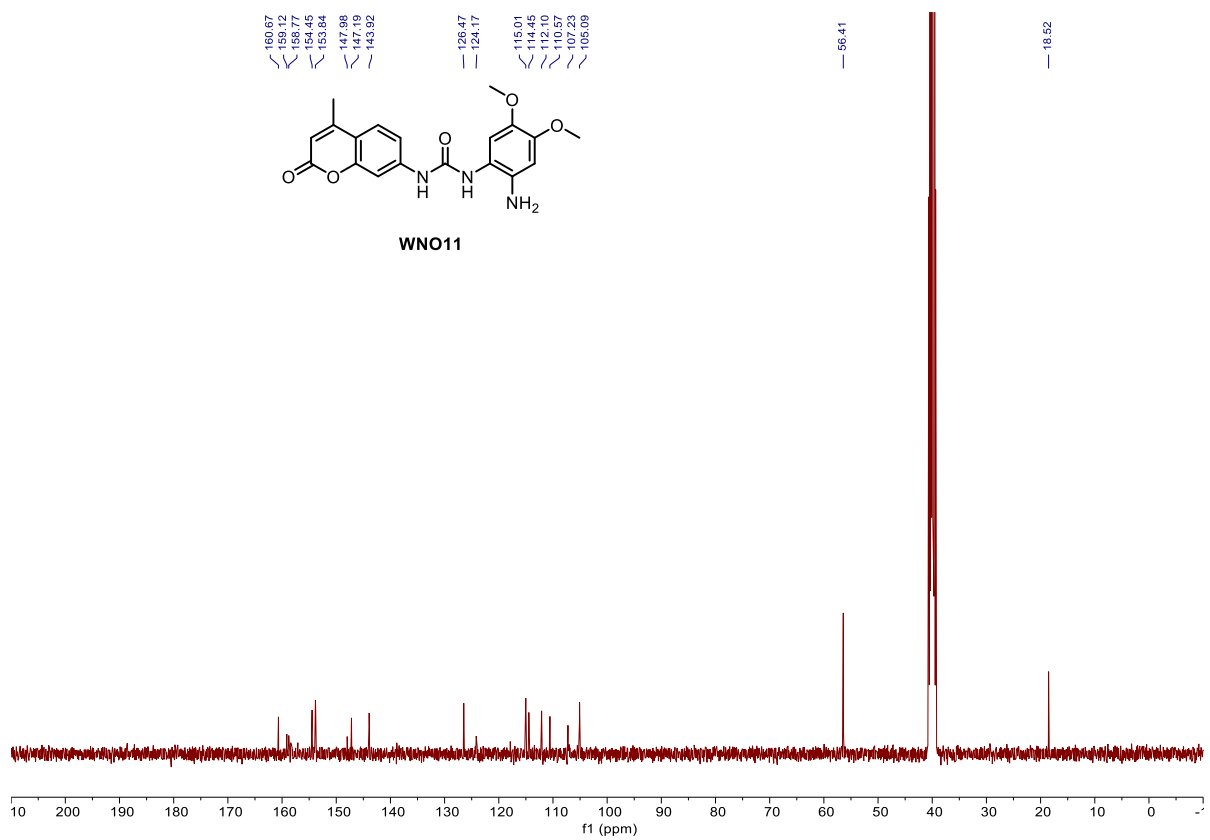
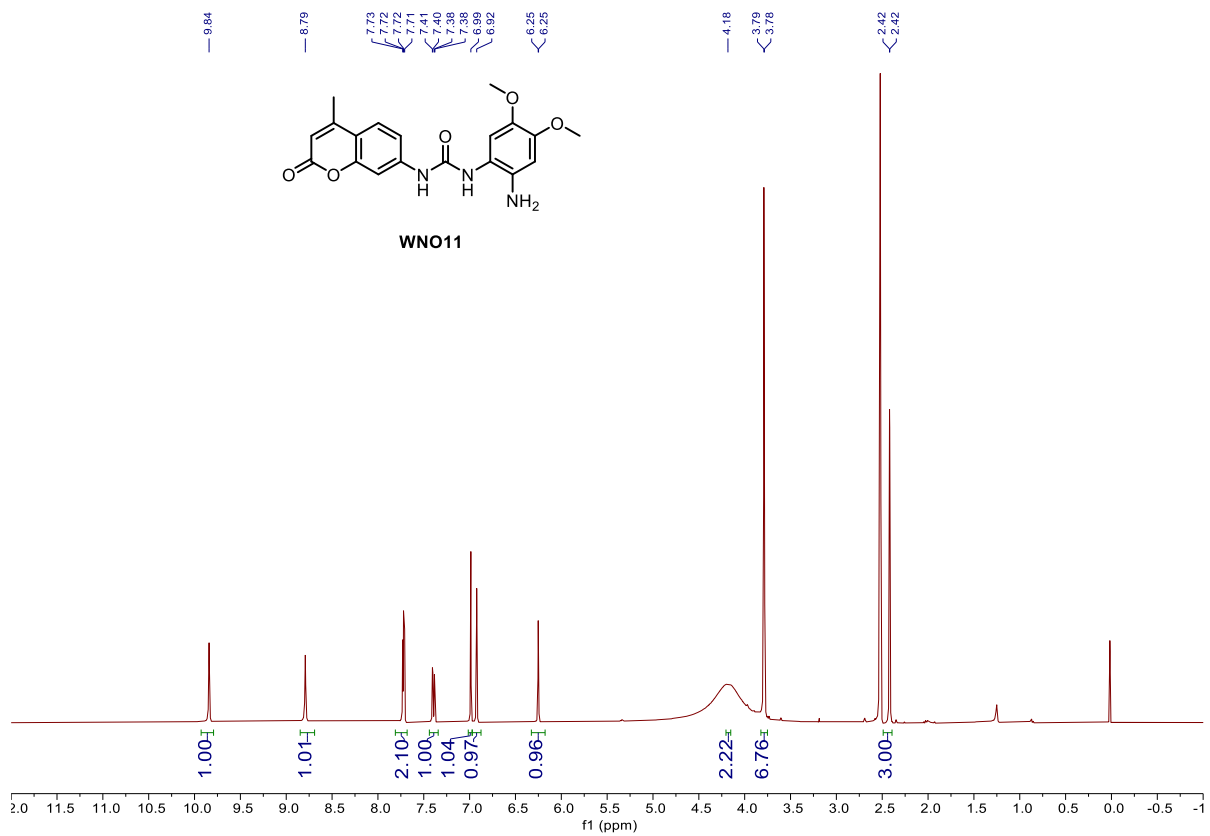


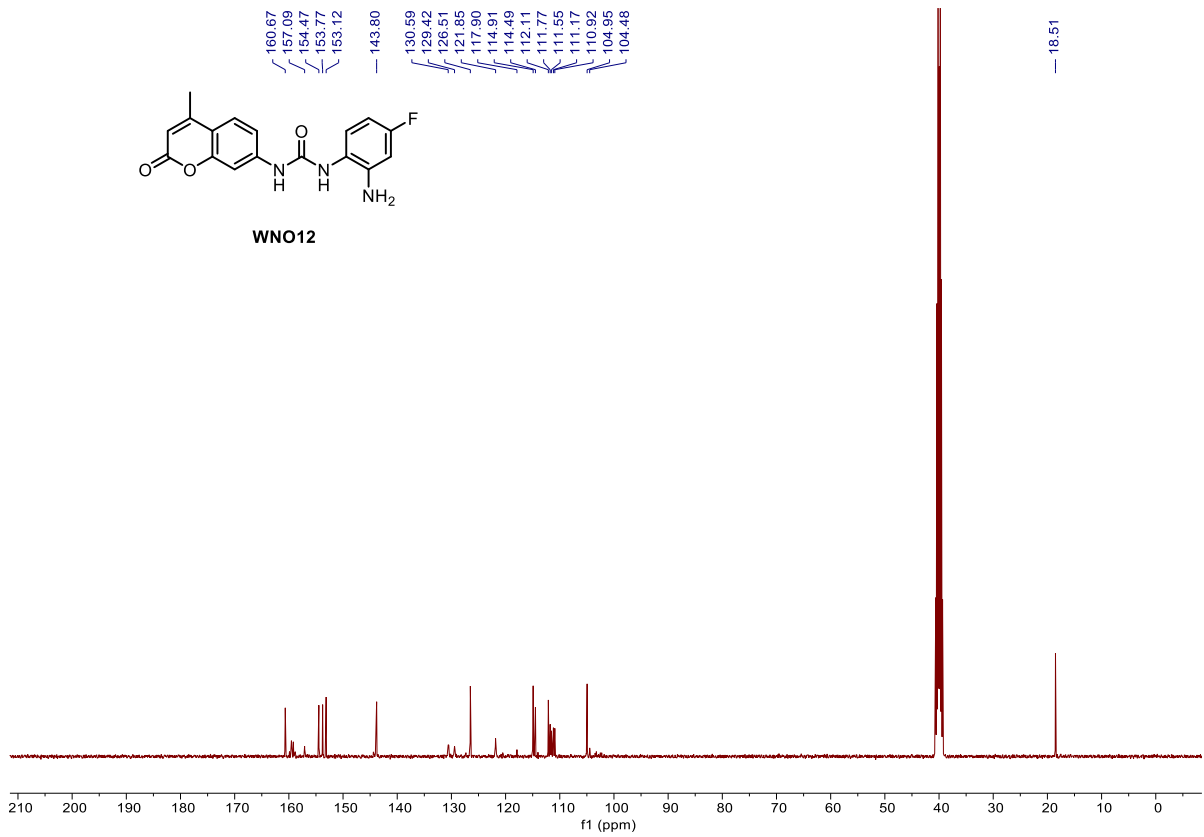
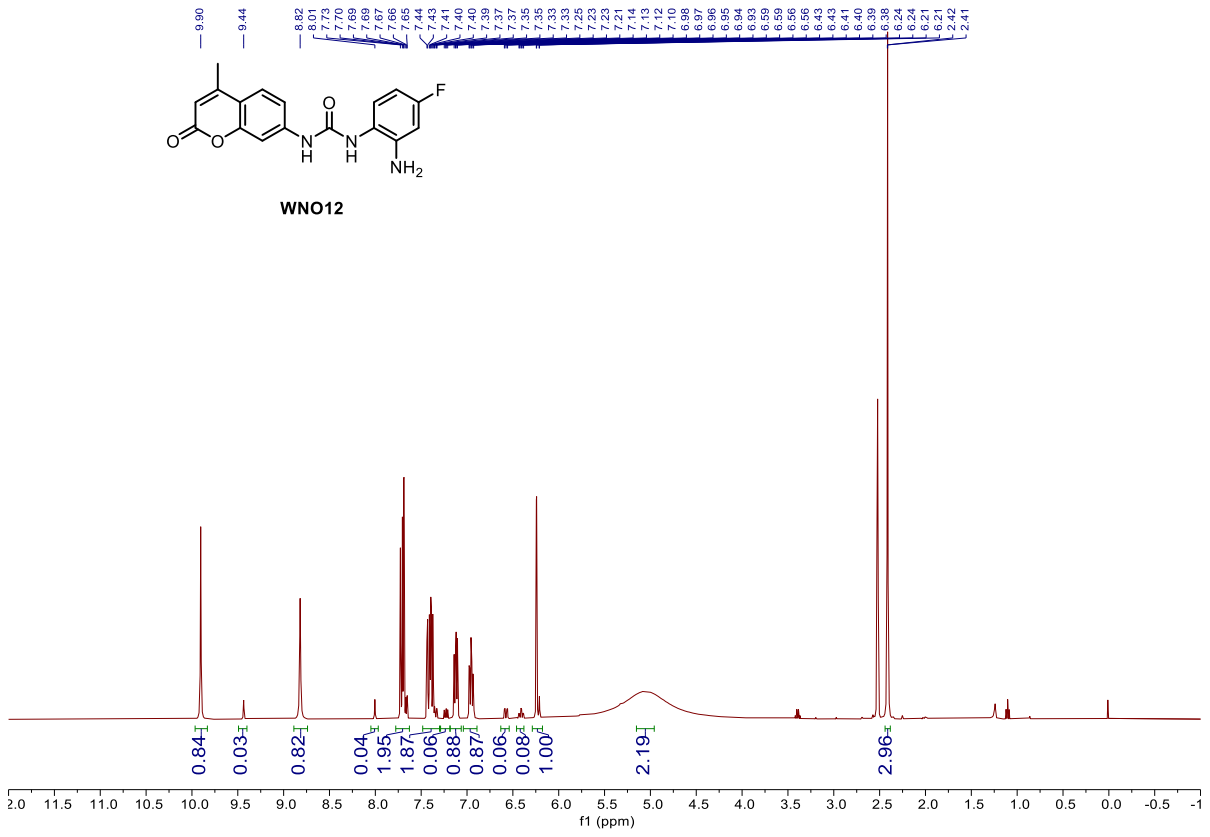
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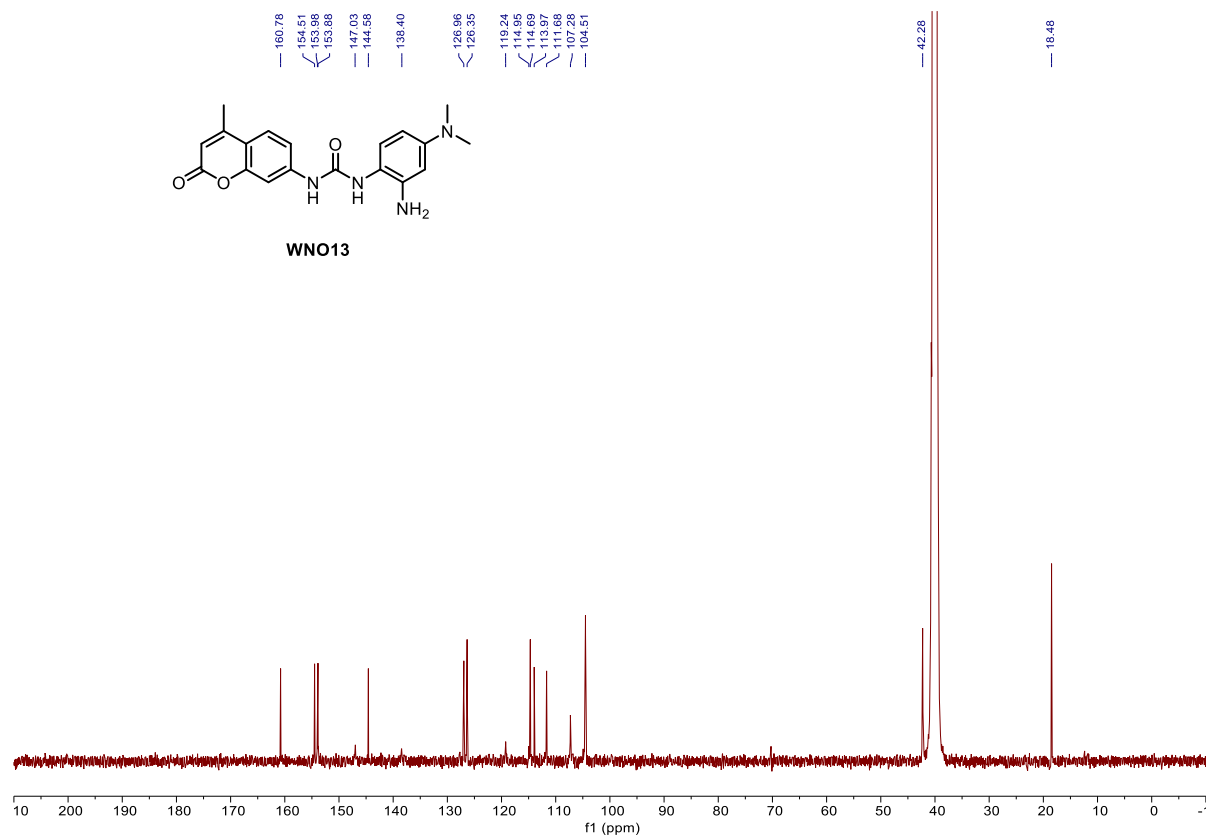
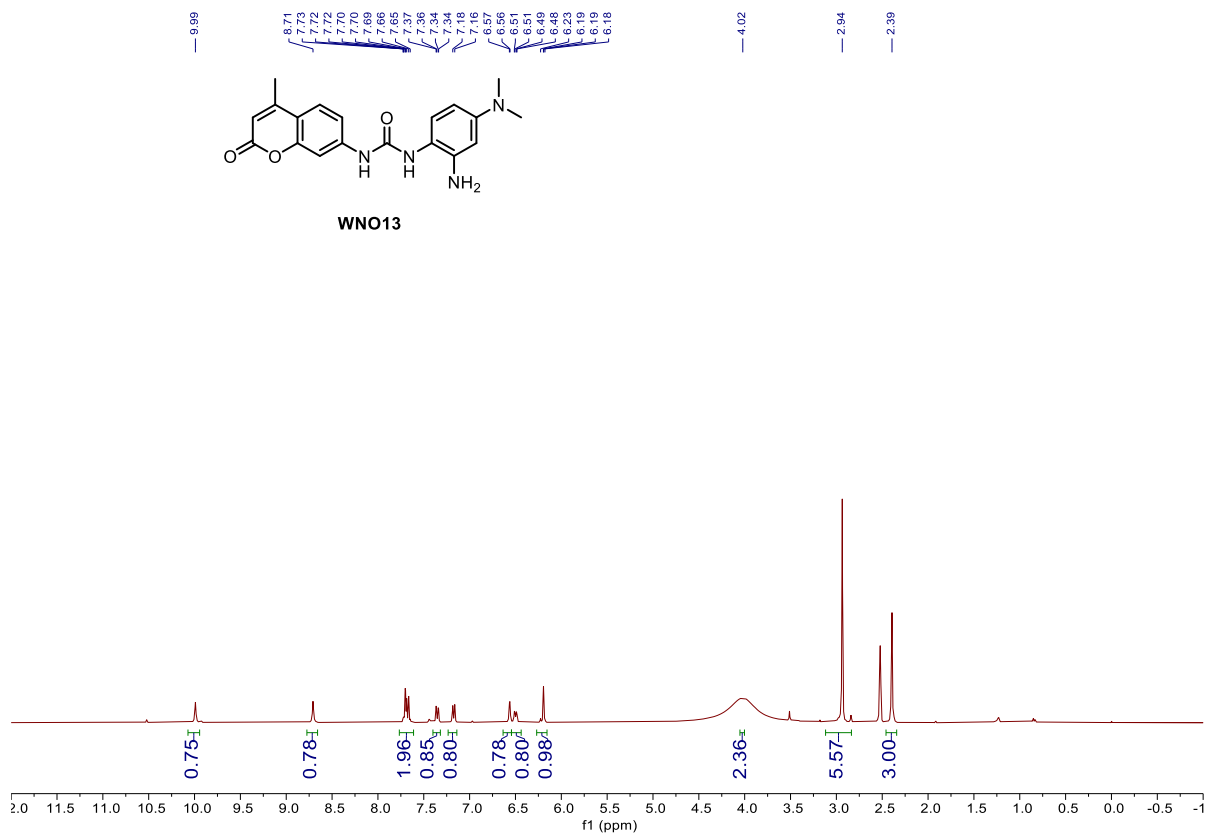


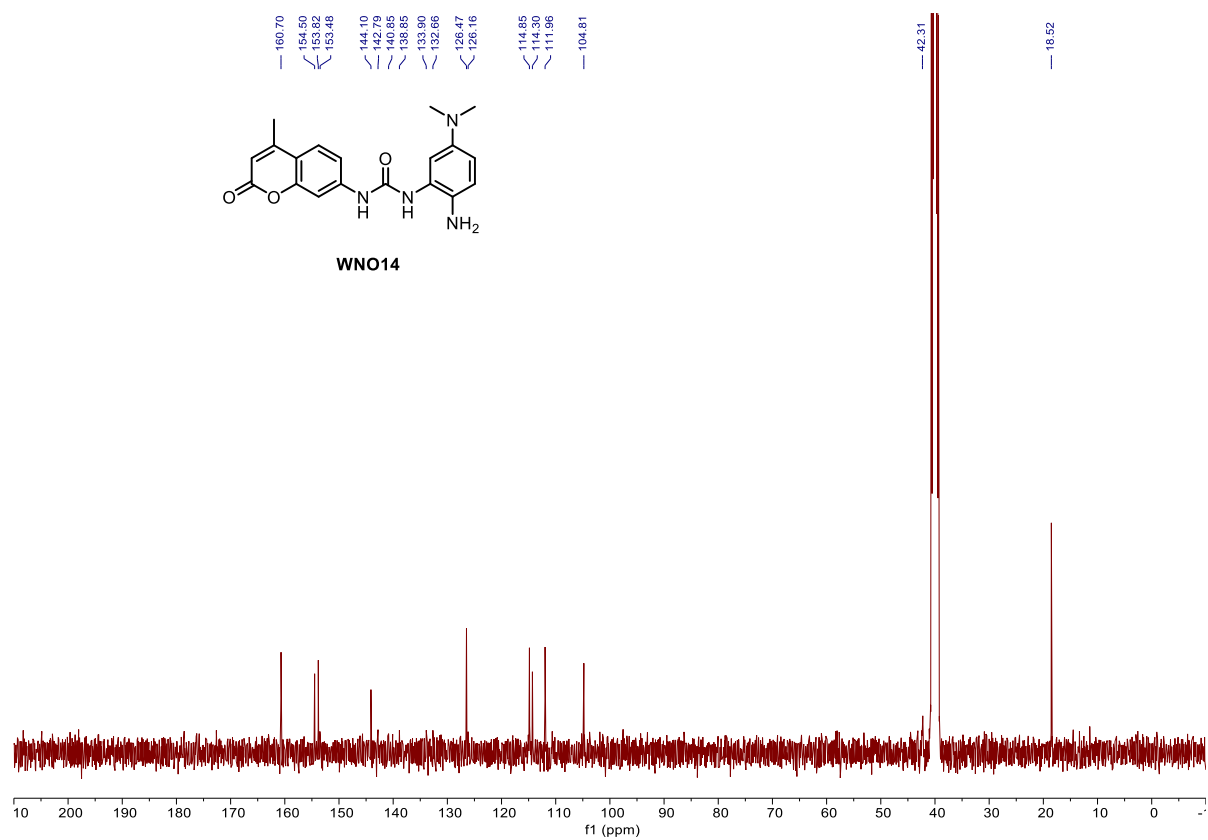
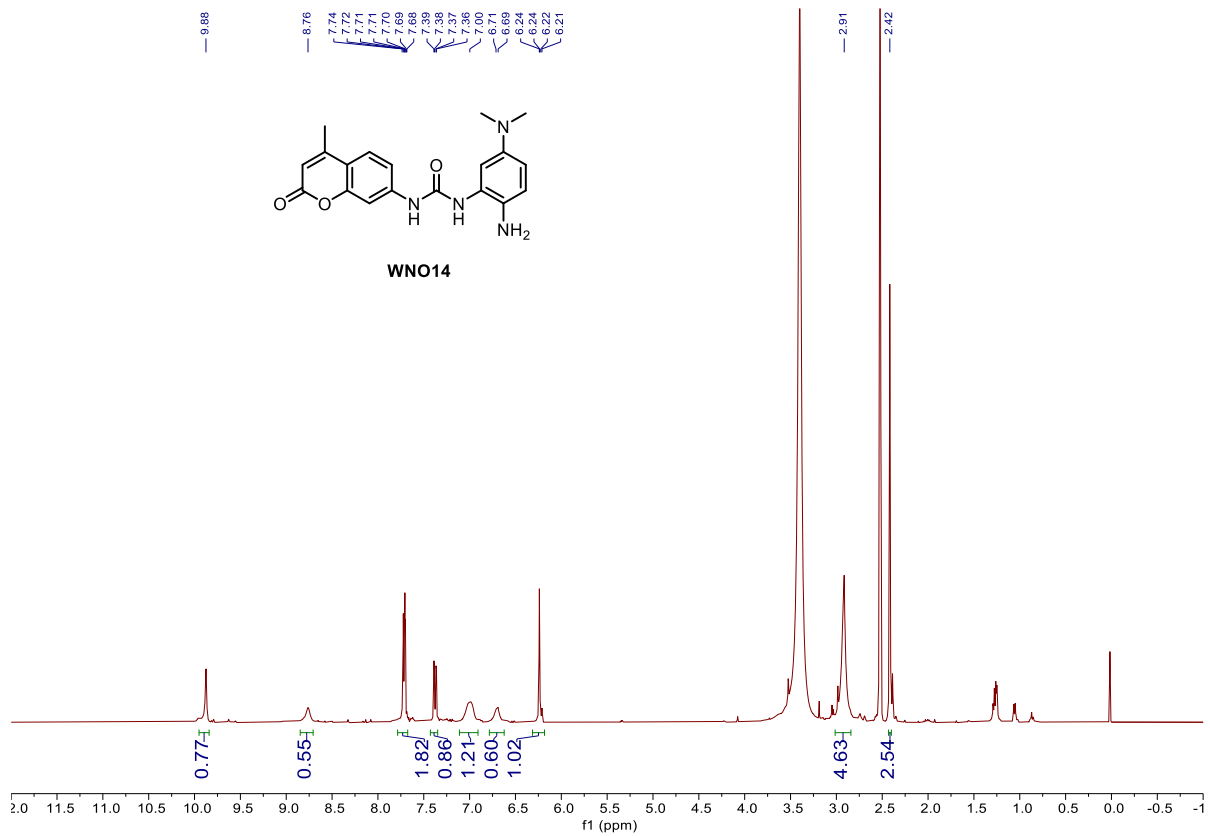
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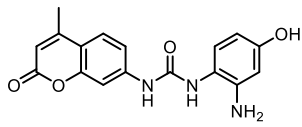




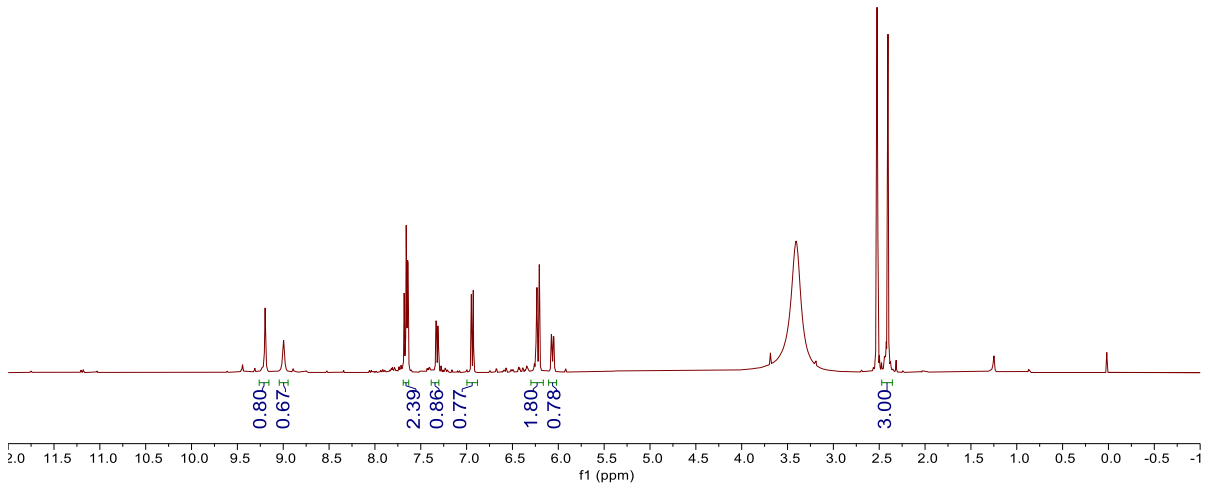




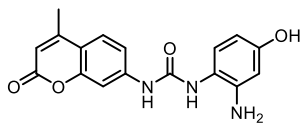
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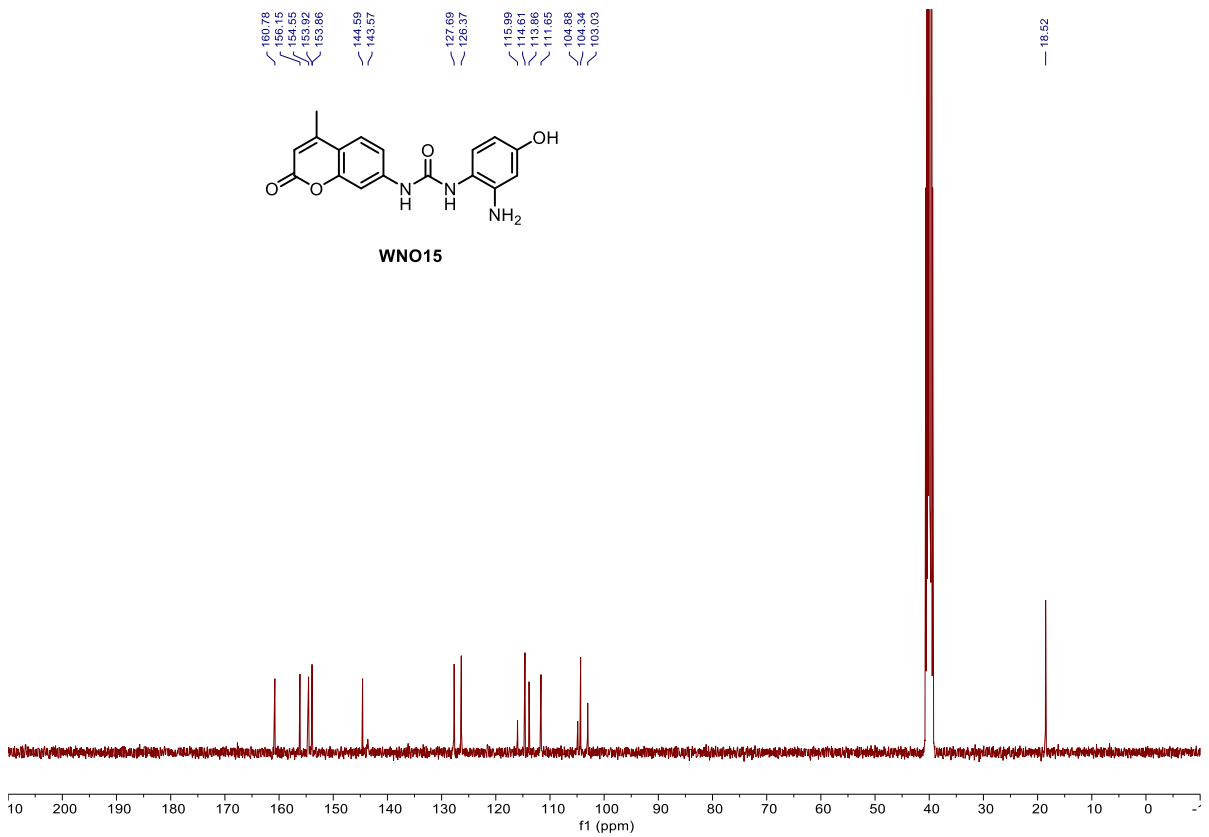
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WNO15



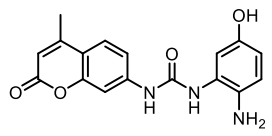
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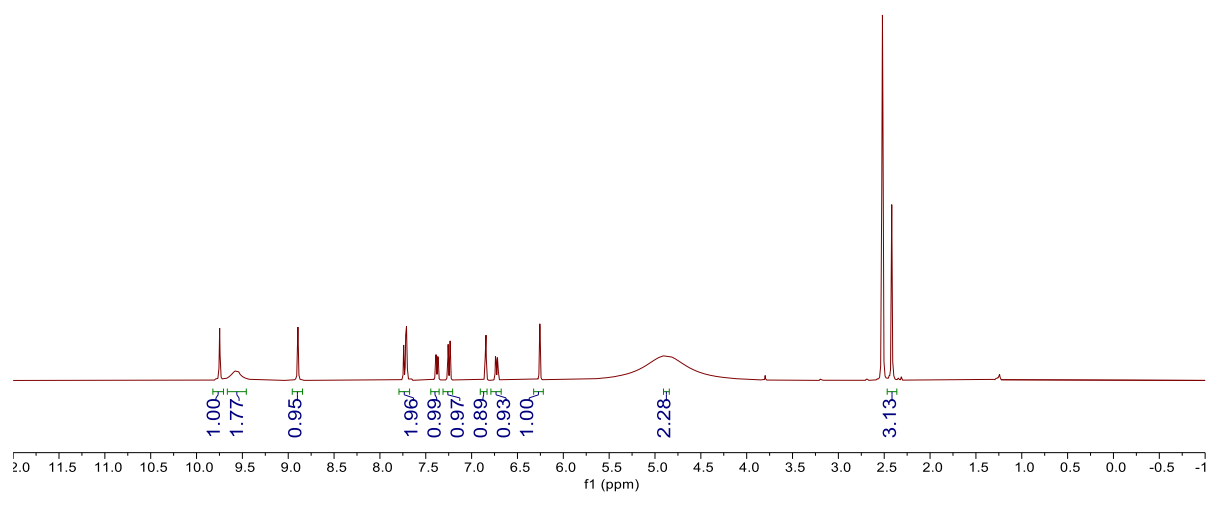
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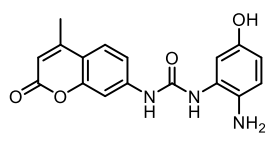
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WNO16

