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Supporting information for

TURN-ON FLUORESCENT PROBES FOR NITRIC OXIDE BASED ON THE ORTHO-HYDROXYAMINO STRUCTURE SHOWING NO INTERFERENCE WITH DEHYDROASCORBIC ACID.

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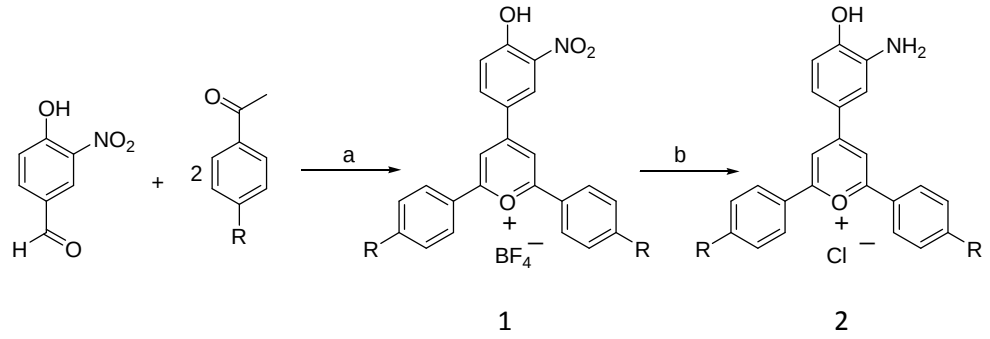
Experimental section

Materials and instruments

All commercially available reagents and solvents were used as received.Deionized water was produced by a Milli-Q water purification system.

Fourier Transform Infrared (FT-IR) spectra were acquired using a FT-IR-6200 type A JASCO spectrometer, with 4 cm^{-1} resolution and 50 scans accumulation. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). A Q-TOF Premier mass spectrometer with an electrospray source (Waters, Manchester, UK) has been used.UV–Vis absorption spectra were recorded in a Hewlett–Packard 8453 apparatus. Steady-state fluorescence spectra were recorded in a Spex Fluorog 3-11 equipped with a 450 W xenon lamp.

Synthetic procedures and characterizations



1 2

Scheme S1 Synthesis of the pyrylium probes **2**. Reagents and conditions: (a) $BF_3 \cdot OEt_2$ in toluene (2h, reflux) (b) $SnCl_2 \cdot H_2O$, HCl in CH_2Cl_2 (3h, reflux).

Compound 1a. 2 equivalents of $BF_3 \cdot OEt_2$ (1.50 ml; 11.98 mmol) were added to a solution of 4-hydroxy-3-nitrobenzaldehyde (1 g; 5.99 mmol) and 2 equivalents of acetophenone (1.39 ml; 11.98 mmol) in anhydrous toluene. The clear solution was refluxed for 2 h. After cooling to room temperature, acetone was added and the solution was poured into excess ether (200ml).

A yellow precipitate was formed which was then filtered, washed with ether and dried under vacuum. Analyses confirmed that the precipitate was the pyrylium salt **1a** (400 mg, 16%).IR (ATR)(cm^{-1}) 3256, 3099, 1601, 1492, 1049; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 9.17 (s, 1H), 9.00 (s, 2H), 8.74 (d, J = 8.8 Hz, 1H), 8.53 (d, J = 7.7 Hz, 4H), 7.83 (t, J = 7.2 Hz, 2H), 7.76 (t, J = 7.5 Hz, 4H), 7.36 (d, J = 8.9 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 169.64, 162.54, 158.45, 139.65, 136.19, 135.37, 130.28, 129.55, 129.04, 128.29, 122.78, 120.66, 114.04; HRMS(ESI-TOF)$^{+}$ calculated for $C_{23}H_{16}NO_4^{+}$ (M^{+})(m/z): 370.1079; experimental (M^{+})(m/z): 370.1081.

Compound 1b. 2 equivalents of $BF_3 \cdot OEt_2$(1.50 ml; 11.98 mmol) were added to a solution of 4-hydroxy-3-nitrobenzaldehyde (1 g; 5.99 mmol) and 2 equivalents 4-methylacetophenone (1.66 ml; 11.98 mmol) in anhydrous toluene. The clear solution was refluxed for 2 h. After cooling to room temperature, acetone was added and the solution was poured into excess ether (200ml).

A yellow precipitate was formed which was then filtered, washed with ether and dried under vacuum. Analyses confirmed that the precipitate was the pyrylium salt **1b** (650 mg, 23%). IR (ATR)(cm^{-1}) 3249, 3108, 1600, 1495, 1248; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 9.14 (s, 1H), 8.92 (s, 2H), 8.70 (d, *J* = 9.4 Hz, 1H), 8.42 (d, *J* = 6.6 Hz, 4H), 7.58 (d, *J* = 6.6 Hz, 4H), 7.31 (d, *J* = 8.9 Hz, 1H), 2.08 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 169.19, 161.83, 158.29, 146.72, 139.51, 136.02, 130.93, 128.86, 128.06, 126.62, 122.76, 120.56, 112.85, 21.99; HRMS (ESI-TOF)$^{+}$ calculated for $C_{25}H_{20}NO_4^{+}$ (M^{+})(m/z): 398,1392; experimental (M^{+})(m/z): 398,1393

Compound 1c. 2 equivalents of $BF_3 \cdot OEt_2$(1.50 ml; 11.98 mmol) were added to a solution of 4-hydroxy-3-nitrobenzaldehyde (1 g; 5.99 mmol) and 2 equivalents of 4-methoxyacetophenone (1.78 ml; 11.98 mmol) in anhydrous toluene. The clear solution was refluxed for 2 h. After cooling to room temperature, acetone was added and the solution was poured into excess ether (200ml).

A red precipitate was formed which was then filtered, washed with ether and dried under vacuum. Analyses confirmed that the precipitate was the pyrylium salt **1c** (440mg, 14%). IR (ATR) (cm^{-1}) 3199, 3114, 1597, 1460, 1176; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 9.09 (s, 1H), 8.80 (s, 2H), 8.67 (d, *J* = 6.9 Hz, 1H), 8.49 (d, *J* = 8.9 Hz, 4H), 7.36 (d, *J* = 8.9 Hz, 1H), 7.29 (d, *J* = 8.9 Hz, 4H), 3.97 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 168.40, 165.16, 160.56, 157.50, 139.12, 135.65, 131.11, 127.53, 123.02, 121.39, 120.22, 115.74, 111.31, 56.41; HRMS (ESI-TOF)$^{+}$: calculated for $C_{25}H_{20}NO_6^{+}$ (M^{+})(m/z): 430.1291; experimental (M^{+})(m/z): 430.1291

Compound 1d. 2 equivalents of $BF_3 \cdot OEt_2$(1.50 ml; 11.98 mmol) were added to a solution of 4-hydroxy-3-nitrobenzaldehyde (1 g; 5.99 mmol) and 2 equivalents of 4-phenylacetophenone (2.34 g; 11.98 mmol) in anhydrous toluene. The clear solution was refluxed for 2 h. After cooling to room temperature, acetone was added and the solution was poured into excess ether (200ml).

A red precipitate was formed which was then filtered, washed with ether and dried under vacuum. Analyses confirmed that the precipitate was the pyrylium salt **1d** (504 mg, 14%). IR (ATR) (cm^{-1}) 3076.87, 1600.63, 1488.78, 1403.92, 1323.89; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 9.17 (d, *J* = 2.5 Hz, 1H), 9.00 (s, 2H), 8.72 (dd, *J* = 9.1, 2.5 Hz, 1H), 8.59 (d, *J* = 8.6 Hz, 3H), 8.06 (d, *J* = 8.6 Hz, 3H), 7.86 (d, *J* = 7.2 Hz, 3H), 7.55 (t, *J* = 7.4 Hz, 3H), 7.49 (t, *J* = 7.3 Hz, 2H), 7.28 (d, *J* = 9.0 Hz, 1H);^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 168.57, 161.51, 159.38, 146.33, 139.97, 138.61, 135.97, 129.68, 129.57, 128.54, 128.39, 128.19, 127.62, 122.12, 121.15, 113.48.; HRMS (ESI-TOF)$^{+}$: calculated for $C_{35}H_{24}NO_4^{+}$ (M^{+})(m/z): 522.1705; experimental (M^{+})(m/z): 522.1709

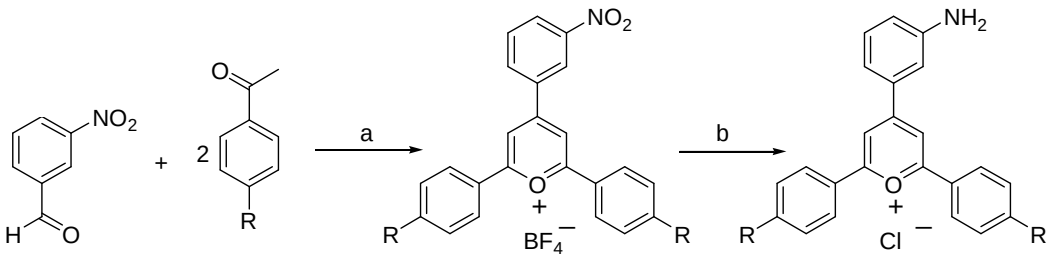
Compound 2a. $SnCl_2 \cdot 2H_2O$ (0.916g; 3.96mmol) was added to a solution of compound **1a** (0.300g; 0.66 mmol) in dichloromethane (30 mL) and excess of hydrochloric acid (0.61 ml; 19.8 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. After this time, the reaction mixture was neutralized with sodium hydroxide and extracted with dichloromethane. Finally, the organic fractions werw collected, dried with Na_2SO_4 and the solvent was removed. A purple powder was obtained (270 mg, 96%). IR(ATR)(cm^{-1}) 3271, 3199, 2921, 2854, 1547, 1461, 1229; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.15 (dd, *J* = 24.4, 6.7 Hz, 4H), 8.03 (d, *J* = 9.6 Hz, 1H), 7.89 (s, 1H), 7.62 (dd, *J* = 15.4, 7.0 Hz, 6H), 7.55 (s, 1H), 7.14 (s, 1H), 6.31 (d, *J* = 9.4 Hz, 1H), 4.89 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ(ppm)

178.54, 157.22, 155.79, 141.75, 140.32, 131.87, 129.61, 125.97, 122.89, 118.97, 106.21, 105.60, 104.89; HRMS (ESI-TOF)$^+$ calculated for $C_{23}H_{18}NO_2^+$ (M^+)(m/z): 340.1338; experimental (M^+)(m/z): 340.1342

Compound 2b. $SnCl_2 \cdot 2H_2O$ (0.86g; 3.72mmol) was added to a solution of compound **1b** (0.300g; 0.62 mmol) in dichloromethane (30 mL) and excess of hydrochloric acid (0,57 ml; 18,6 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. After this time, the reaction mixture was neutralized with sodium hydroxide and extracted with dichloromethane. Finally, the organic fractions were collected, dried with Na_2SO_4 and the solvent was removed. A purple powder was obtained (283 mg, 100%). IR (ATR)(cm^{-1}) 3458, 3318, 3031, 2921, 1607, 1585, 1541, 1506, 1461; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.68 (d, *J* = 7.6 Hz, 4H), 7.51 (d, *J* = 9.2 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 4H), 7.19 (s, 2H), 6.83 (s, 1H), 6.47 (d, *J* = 9.4 Hz, 1H), 2.38 (s, 6H) ; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{22}NO_2^+$ (M^+)(m/z): 368.1651; experimental (M^+)(m/z): 368.1653

Compound 2c. $SnCl_2 \cdot 2H_2O$ (0.81g; 3.48mmol) was added to a solution of compound **1c** (0.300g; 0.58 mmol) in dichloromethane (30 mL) and excess of hydrochloric acid (0.53 ml; 17.4 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. After this time, the reaction mixture was neutralized with sodium hydroxide and extracted with dichloromethane. Finally, the organic fractions were collected, dried with Na_2SO_4 and the solvent was removed. A purple powder was obtained (259 mg, 91%). IR (ATR)(cm^{-1}) 3275, 3121, 2925, 2844, 1600, 1503, 1453, 1241, 1175 ; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.10 (s, 1H), 8.05 (d, *J* = 6.0 Hz, 4H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 4H), 7.10 (s, 2H), 6.23 (d, *J* = 9.3 Hz, 1H), 4.73 (s, 2H), 3.86 (s, 6H) ; ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 161.97, 159.69, 157.18, 142.24, 141.54, 130.99, 127.87, 124.18, 122.15, 117.81, 115.04, 105.49, 104.29, 55.98; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{22}NO_4^+$ (M^+)(m/z): 400.1549 ; experimental (M^+)(m/z): 400.1552

Compound 2d. $SnCl_2 \cdot 2H_2O$ (0.688 g; 2.94 mmol) was added to a solution of compound **1d** (0.300g; 0.49 mmol) in dichloromethane (30 mL) and excess of hydrochloric acid (0.45 ml; 14.7 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. After this time, the reaction mixture was neutralized with sodium hydroxide and extracted with dichloromethane. Finally, the organic fractions were collected, dried with Na_2SO_4 and the solvent was removed. A purple powder was obtained (260 mg, 100%). IR (ATR)(cm^{-1}) 3398.92, 3278.39, 3032.51, 2917.77, 2851.24, 1643.05, 1548.56, 1460.81, 1402, 1385.6, 1351.86; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.20 (dd, *J* = 27.7, 6.9 Hz, 1H), 8.01 (d, *J* = 9.7 Hz, 1H), 7.89 (t, *J* = 10.7 Hz, 1H), 7.78 (d, *J* = 7.2 Hz, 1H), 7.56 (s, 1H), 7.51 (s, 1H), 7.42 (dd, *J* = 4.7, 2.3 Hz, 1H), 7.23 (t, *J* = 6.8 Hz, 1H), 7.19 – 7.07 (m, 2H), 6.28 (d, *J* = 7.6 Hz, 1H), 4.86 (s, 1H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 178.51, 156.91, 155.48, 142.88, 142.55, 141.79, 140.32, 139.43, 139.37, 137.78, 131.53, 130.85, 130.79, 129.54, 129.52, 129.33, 128.63, 128.58, 127.72, 127.58, 127.28, 127.24, 126.58, 126.54, 125.74, 122.88, 119.11, 106.28, 105.64, 104.93.; HRMS (ESI-TOF)$^+$ calculated for $C_{35}H_{26}NO_2^+$ (M^+)(m/z): 492.1964 ; experimental (M^+)(m/z): 492.1969



3 **4**

Scheme S2 Synthesis of the pyrylium salts **3** and **4** (R=OMe). Reagents and conditions: (a) $BF_3 \cdot OEt_2$ in toluene (2h, reflux) (b) $SnCl_2 \cdot H_2O$, HCl in CH_2Cl_2 (3h, reflux).

Model compound 3. 2 equivalents of $BF_3 \cdot OEt_2$ (1.67 ml; 13.34 mmol) were added to a solution of 3-nitrobenzaldehyde (1 g; 6.67 mmol) and 2 equivalents of 4-methoxyacetophenone(1.99 g; 13.34 mmol) in anhydrous toluene. The clear solution was refluxed for 2 h. After cooling to room temperature, acetone was added and the solution was poured into excess ether (200ml).

A red precipitate was formed which was then filtered, washed with ether and dried under vacuum. Analyses confirmed that the precipitate was the pyrylium salt **3** (337mg, 10%).IR(ATR)(cm^{-1})3103.87,2929.34, 2847.38, 1600.63, 1488.78, 1349.93 ; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 9.22 (s, 1H), 8.97 (s, 2H), 8.83 (d, *J* = 7.7 Hz, 1H), 8.58 (d, *J* = 8.1 Hz, 1H), 8.53 (d, *J* = 8.7 Hz, 3H), 8.02 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 3H), 3.96 (s, 5H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 169.85, 165.59, 161.45, 149.20, 135.99, 134.97, 131.75, 131.56, 128.55, 124.38, 121.62, 116.04, 115.99, 114.01, 56.61, 56.58; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{20}NO_5^+$ (M^+)(m/z): 414.1341; experimental (M^+)(m/z): 414.1343

Model compound 4. $SnCl_2 \cdot 2H_2O$ (0.836 g; 3.60 mmol) was added to a solution of compound **1e** (0.300g; 0.60 mmol) in dichloromethane (30 mL) and excess of hydrochloric acid (0.55 ml; 18 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. After this time, the reaction mixture was neutralized with sodium hydroxide and extracted with dichloromethane. Finally, the organic fractions were collected, dried with Na_2SO_4 and the solvent was removed. A brown powder was obtained (50 mg, 20%). IR(ATR)(cm^{-1})3374.82, 3060.48, 2930.31, 2842.56, 1601.59, 1506.13, 1459.85; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.00 (dd, *J* = 18.0, 8.8 Hz, 1H), 7.44 (s, 1H), 7.04 (dd, *J* = 15.0, 8.9 Hz, 1H), 6.86 – 6.74 (m, 1H), 6.60 (d, *J* = 9.2 Hz, 1H), 5.13 (s, 1H), 4.72 (s, 1H), 3.84 (d, *J* = 8.6 Hz, 1H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 169.85, 165.59, 161.45, 149.20, 135.99, 134.97, 131.75, 131.56, 128.55, 124.38, 121.62, 116.04, 115.99, 114.01, 56.61, 56.58; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{22}NO_3^+$ (M^+)(m/z):384.1600; experimental (M^+)(m/z): 384.1604

Reaction product of 2a with NO/O_2. The reaction product of **2a** with NO/O_2 was isolated after bubbling excess of NO gas in a solution of **2a** (50 mg, 0.12 mmol) in ethanol and precipitate it after pouring into excess ether. A brown powder is obtained (49 mg, 95%) IR (ATR)(cm^{-1}) 3444.24, 3067.23, 2186.88, 1628.59, 1590.99, 1570.74, 1517.7, 1488.78, 1353.78; ^{1}H NMR (500 MHz, MeOH-d_4) δ(ppm) 9.14 (d, *J* = 2.6 Hz, 1H), 8.73 (s, 1H), 8.63 (s, 2H), 8.38 (d, *J* = 3.2 Hz, 2H), 8.37 – 8.32 (m, 5H), 7.76 – 7.71 (m, 3H), 7.68 (t, *J* = 7.5 Hz, 5H).^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 168.85, 166.20, 163.93, 134.90, 134.05, 130.20, 129.85, 128.83, 123.31, 117.61, 113.08;HRMS (ESI-TOF)$^+$ calculated for (M^+)(m/z): 325.1229 ; experimental (M^+)(m/z): 325.1233 .

Fluorometric studies

Preparation of NO stock solutions. Gaseous nitric oxide (NO) was synthesized by a redox reaction between KI (1 M) and $NaNO_2$ (1 M) catalyzed by acid, according to the following reaction.[1]

$$2NO_2^-(aq) + 2I^-(aq) + 4H^+(aq) \rightarrow 2NO(g) + I_2(s) + 2H_2O(l)$$

In this reaction, sodium nitrite is reduced to nitric oxide and potassium iodide is oxidized to iodine in the presence of concentrated sulfuric acid.

DEA/NONOate (1,1-Diethyl-2-hydroxy-2-nitroso-sodium hydrazine, a commercially available NO donor) was purchased from Aldrich. A 15 mM NO stock solution of DEA/NONOate was prepared in 0.01M NaOH solution.

Fluorescence measurements. *NO titrations in aerated water*. Compounds **2 a-d** were dissolved in deionized water to obtain 10 μM solutions. These solutions were titrated by adding increasing volumes of 15mM NO stock solutions of DEA/ NONOate. pH values were adjusted with hydrogen chloride and sodium hydroxide to the interval 7.2-7.4. The fluorescence intensities of the NO reaction products were measured after 5 minutes of reaction at the corresponding excitation wavelength for each compound.

The same procedure was followed to study the behavior of model compound **4** in the presence of 50 equivalents of NO.

An appropriate analysis of the fluorescence intensities versus NO concentration provided us detection limit values. The limit of detection, expressed as the concentration, c_L, is derived from the smallest measure, x_L , that can be detected with reasonable certainty for a given analytical procedure.[2] The value of x_L is given by the equation:

$$x_L = \bar{x}_0 + k\sigma$$

where $\bar{x}_0$ is the mean of the blank measurements, σ is the standard deviation of the blank measurements, and k is a numerical factor chosen according to the confidence level desired. In general, limit of detection (LOD) is the point at which the signal equals three times the noise.($k = 3$)

Selectivity studies. All the fluorescent tests were performed in deionized water adjusting the pH at 7.2-7.4 with hydrogen chloride and sodium hydroxide. The fluorescence intensities were monitored after reacting 10 μM solutions of compounds **2a-d** with 50 equivalents of reactive oxygen species (ROS), reactive nitrogen species (RNS), ascorbic acid (HAA) and dehydroascorbic acid (DHA).

The aqueous solutions of $NaNO_2$ and $NaNO_3$ were freshly prepared and used as nitrite (NO_2^-) and nitrate (NO_3^-) sources, respectively. Hydrogen peroxide (H_2O_2) was diluted promptly from

[1] K.A. Mowery, M.E. Meyerhoff, *Polymer*, **1999**, 40, 6203.

[2] IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8.

the 50 % H_2O_2 solution. Singlet oxygen ($^1O_2$) was originated from the reaction between hydrogen peroxide and hypochlorite in deionized water. [3] Aqueous solution of NaClO and KO_2 were used as a ClO^- and O_2^- source, repectively. Hydroxyl radical (·OH) was produced by the reaction of 1 mM H_2O_2 with 100 μM Fe^{2+} (Fenton's reaction). [4] Aqueous solutions of AA and DHA were freshly prepared before use. The aqueous solution of Piloty's acid was used as a nitroxyl (HNO) source after two hours of reaction. [5] The concentration of the $ONOO^-$ stock solution was calculated by using its molar extinction coefficient of 1670 $M^{-1}cm^{-1}$ at 302 nm.[6] *Synthesis of peroxynitrite.*[7] Briefly, the reaction were performed in mixed solvents of IPA (20 % v/v) and water that also contained 0.2 M isoamyl nitrite, 0.2 M H_2O_2, and 0.3 M NaOH. All incubations were maintained at 25 ºC , and the contents were stirred constantly throughout the course of the reaction. To estimate the amount of peroxynitrite, the reaction mixtures were diluted 400 to 1000 fold using 0.1N NaOH and the absorbance was read at 302 nm. The solutions of peroxynitrite prepared in the homogeneous solvent system were extracted with 4x2 volumes of dichlorometane. MnO_2 is added to the aqueous phase and following decomposition of unreacted H_2O_2 it can be removed by filtration.

[3] Maetzke, A.; Knak Jensen, S. J., *Chem. Phys. Lett.* **2006,** 425, 40-43.
[4] Zheng, H.; Shang, G.-Q.; Yang, S.-Y.; Gao, X.; Xu, J.-G., *Org. Lett.* **2008,** 10, 2357
[5] Cline, M. R.; Toscano, J. P., *J. Phys. Org. Chem.* **2011,** 24, 993
[6] Miyamoto, S.; Martinez, G. R.; Martins, A. P. B.; Medeiros, M. H. G.; Di Mascio, P., *J. Am. Chem. Soc.* **2003,** 125, 4510
[7] Uppu, R. M., *Analytical Biochemistry* **2006,** 354, 165

NMR, FTIR,HRMS spectra
Compound 1a

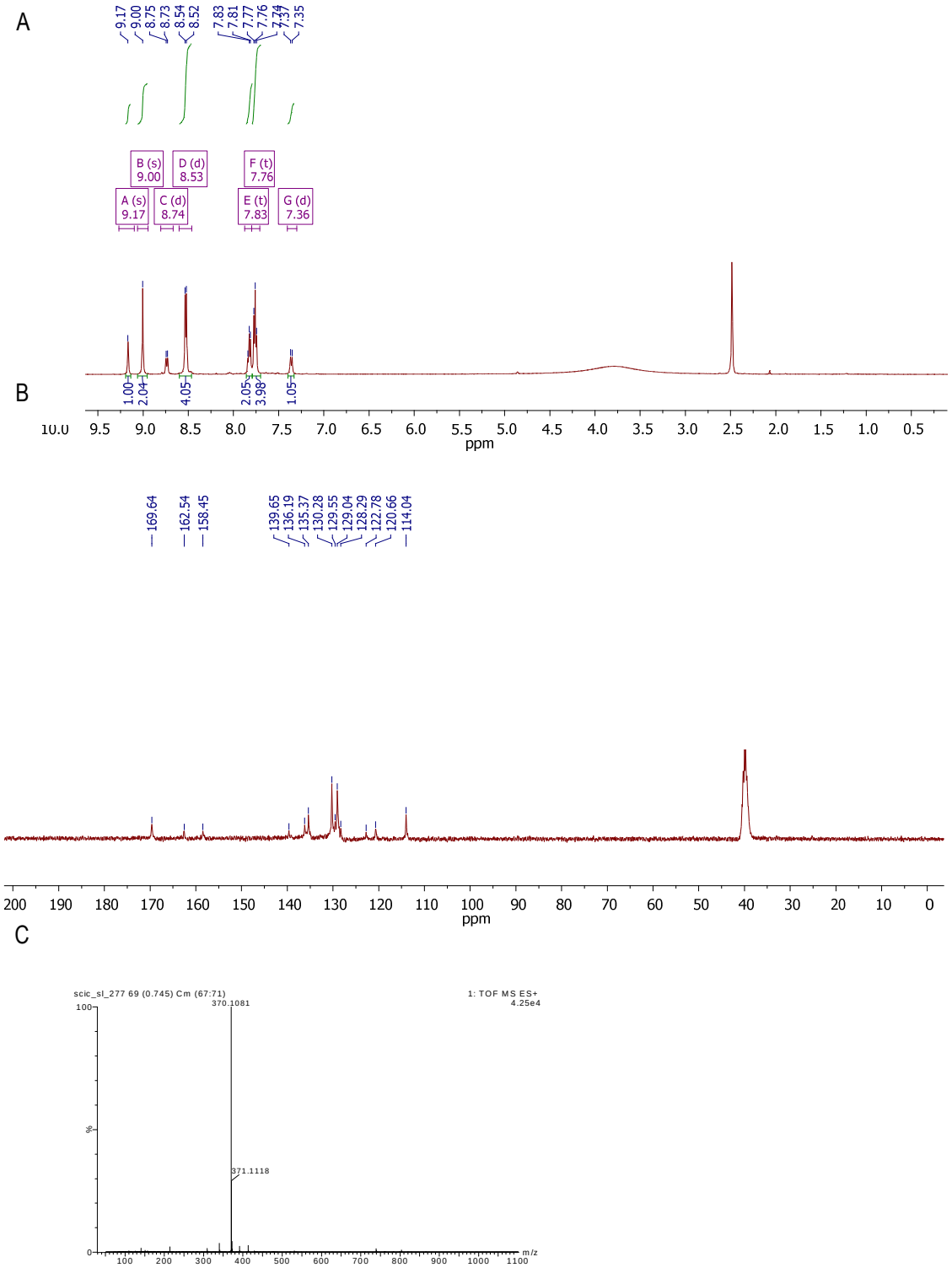


Figure S1. A) ^{1}H NMR (DMSO-d_6) spectra of compound **1a**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **1a**; C) HRMS spectra of compound **1a**

Compound 1b

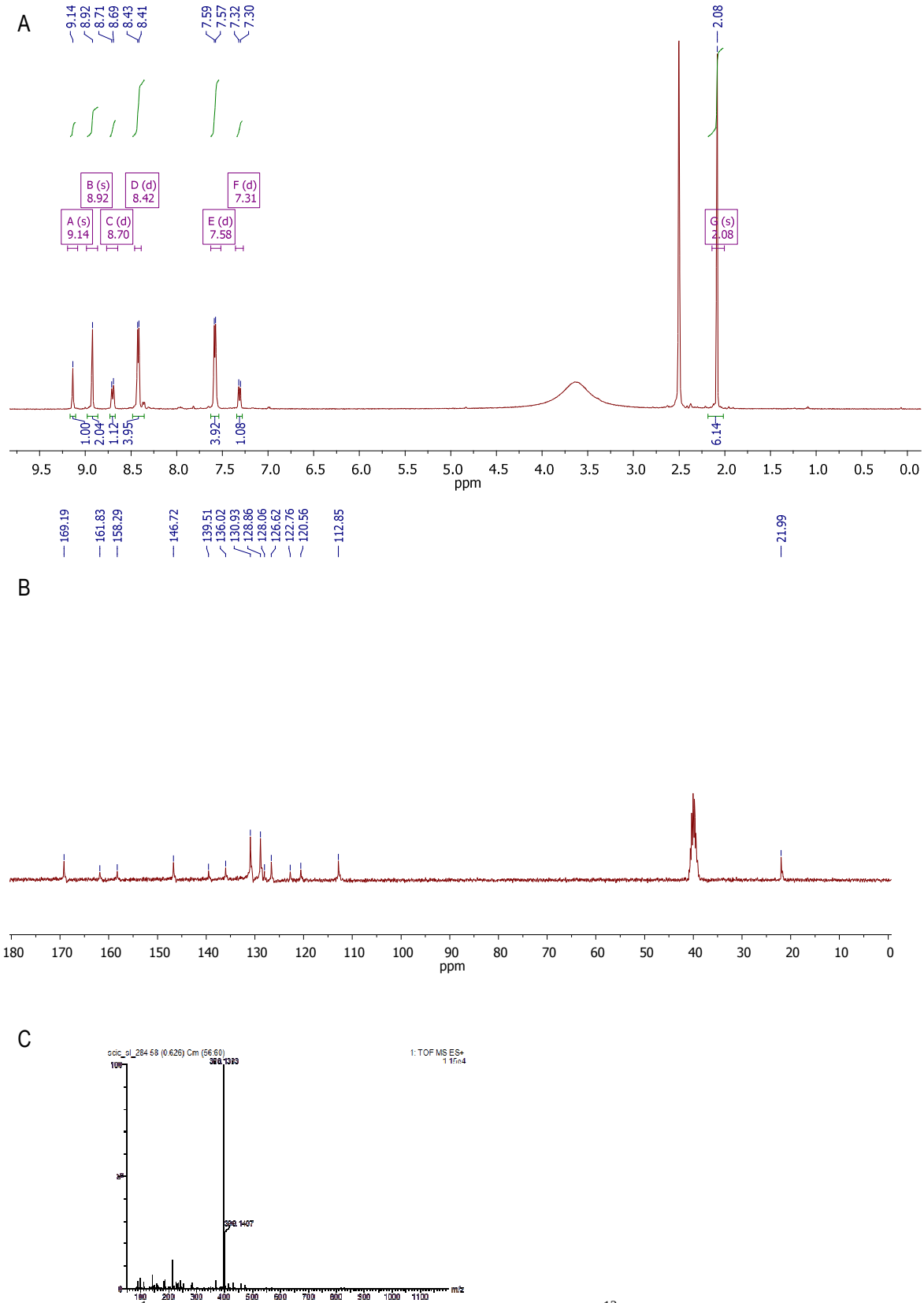


Figure S2. A) ^{1}H NMR (DMSO-d_6) spectra of compound **1**b; B) ^{13}C NMR (DMSO-d_6) spectra of compound **1b**; C) HRMS spectra of compound **1b**

Compound 1c

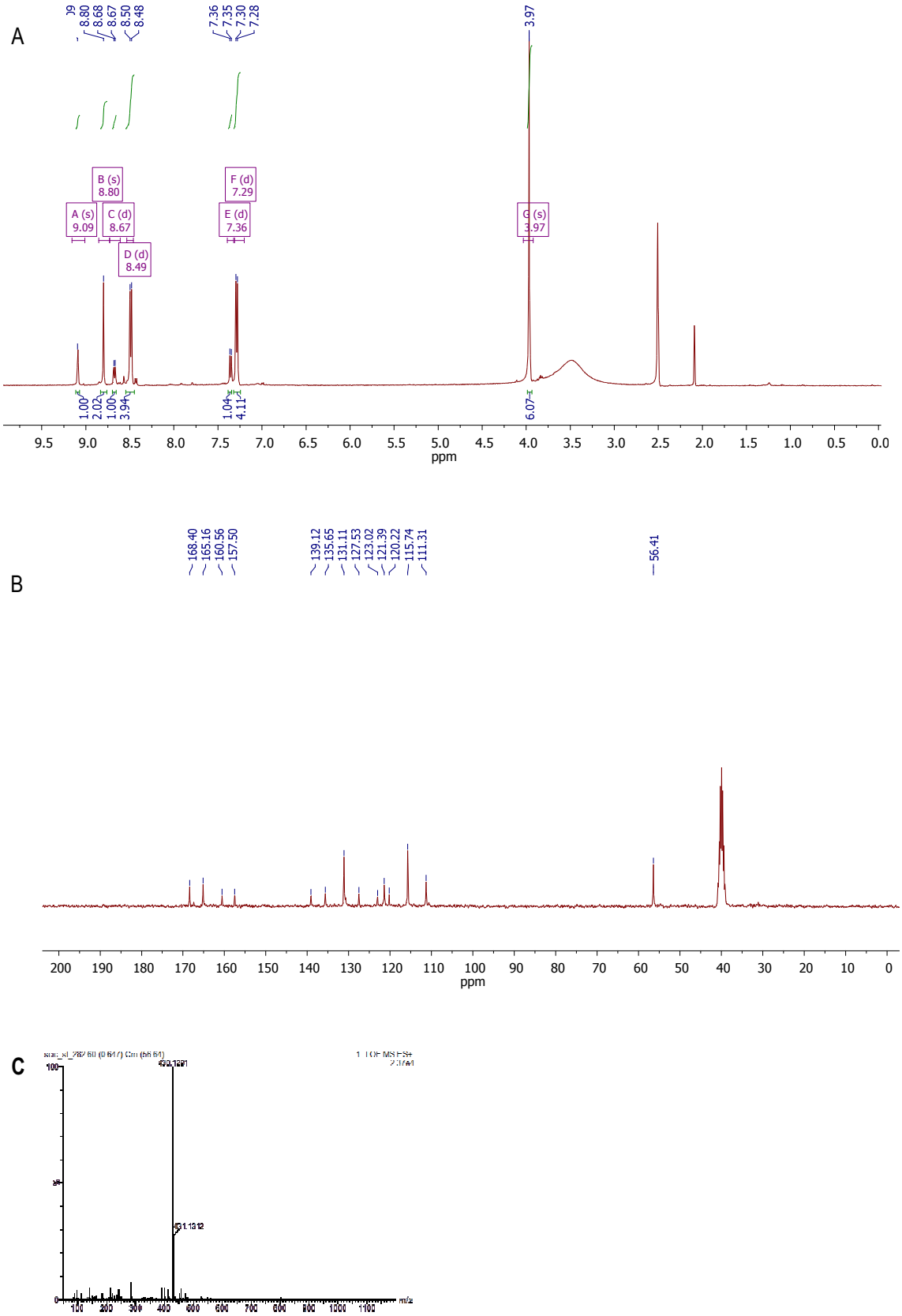


Figure S3. A) ^{1}H NMR (DMSO-d_6) spectra of compound **1c**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **1c**; C) HRMS spectra of compound **1c**.

Compound 1d

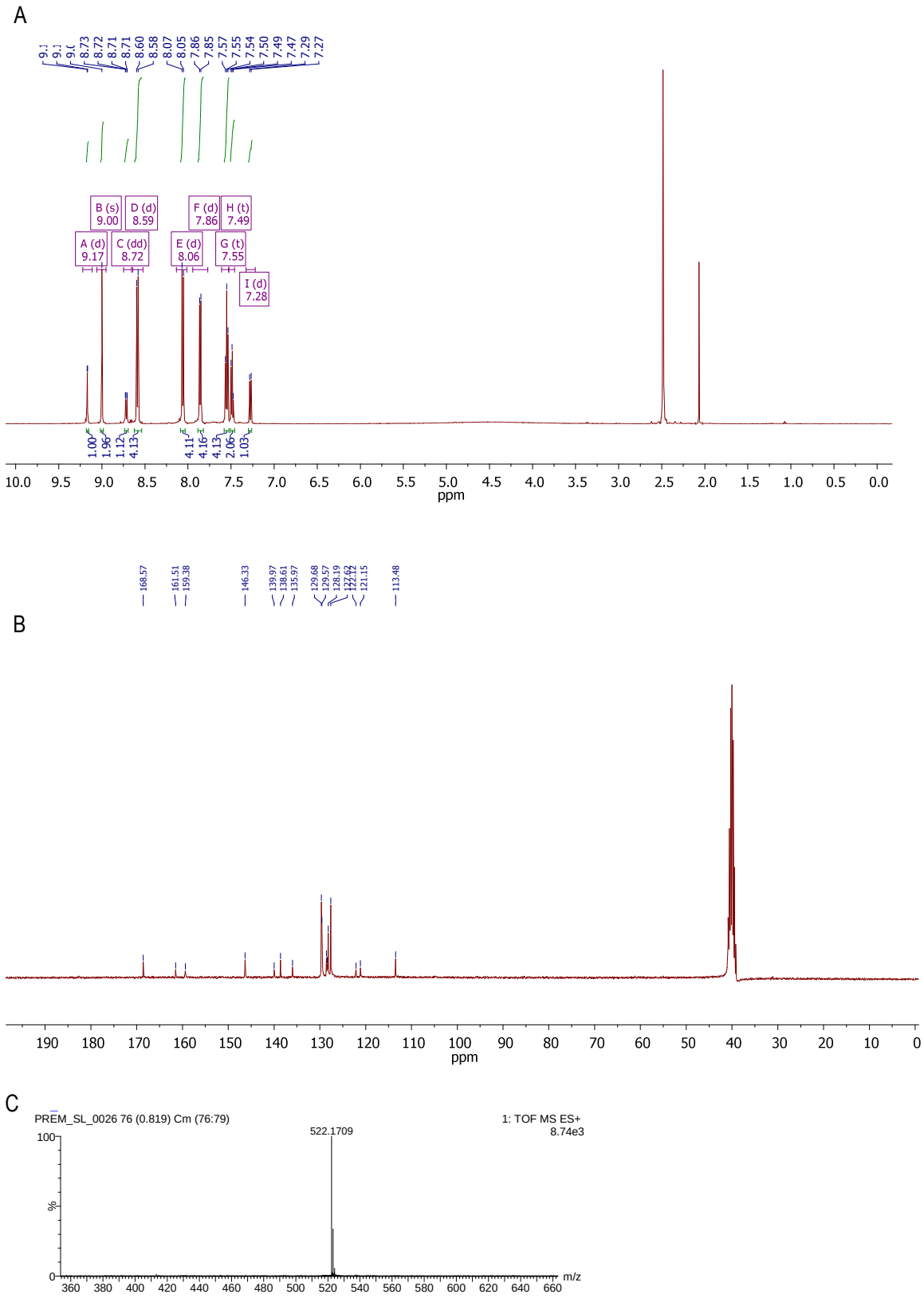


Figure S4. A) ^{1}H NMR (DMSO-d_6) spectra of compound **1d**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **1d**; C) HRMS spectra of compound **1d**

Compound 2a

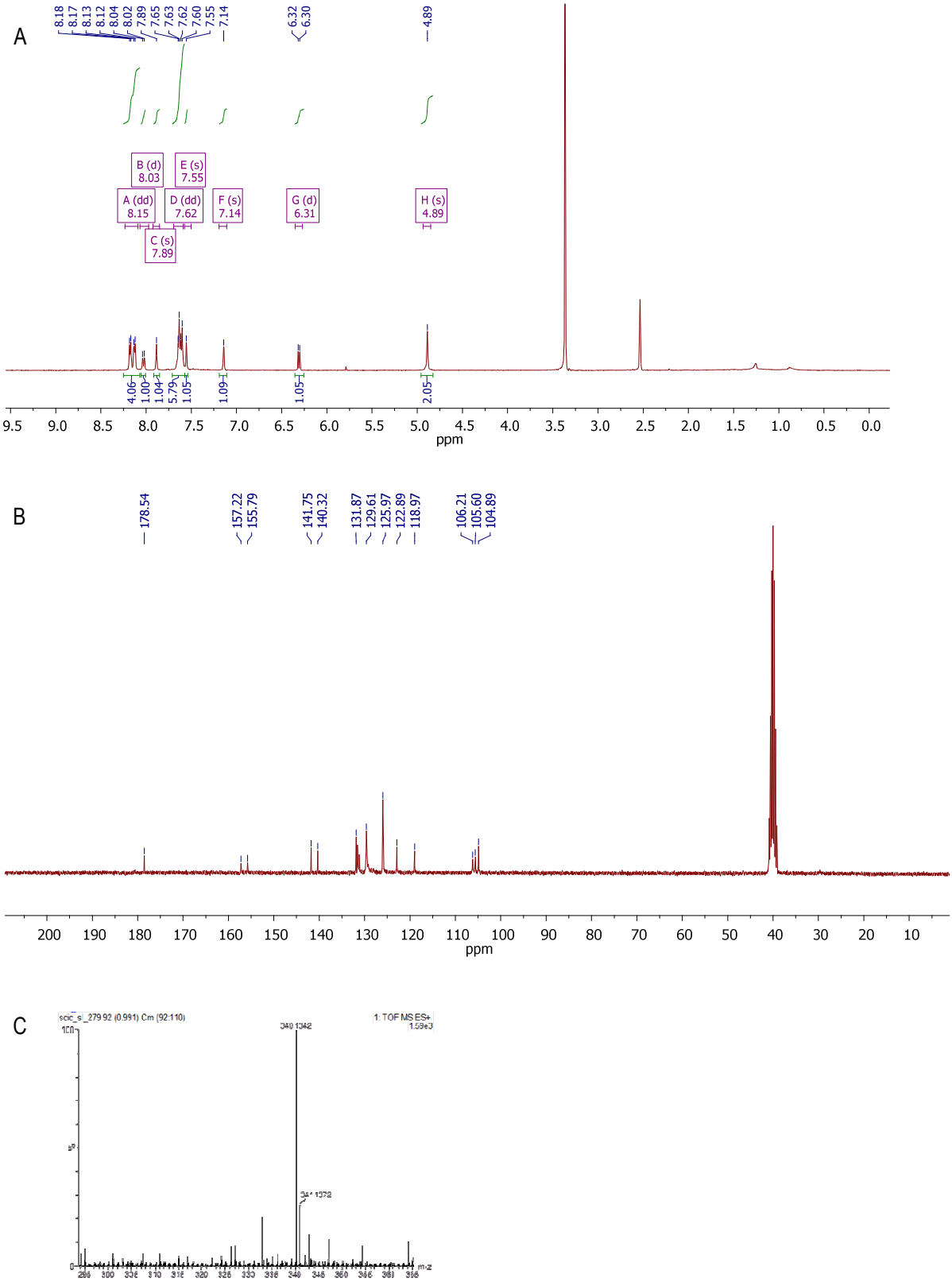


Figure S5. A) ^{1}H NMR (DMSO-d_6) spectra of compound **2a**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **2a**; C) HRMS spectra of compound **2a**

Compound 2b

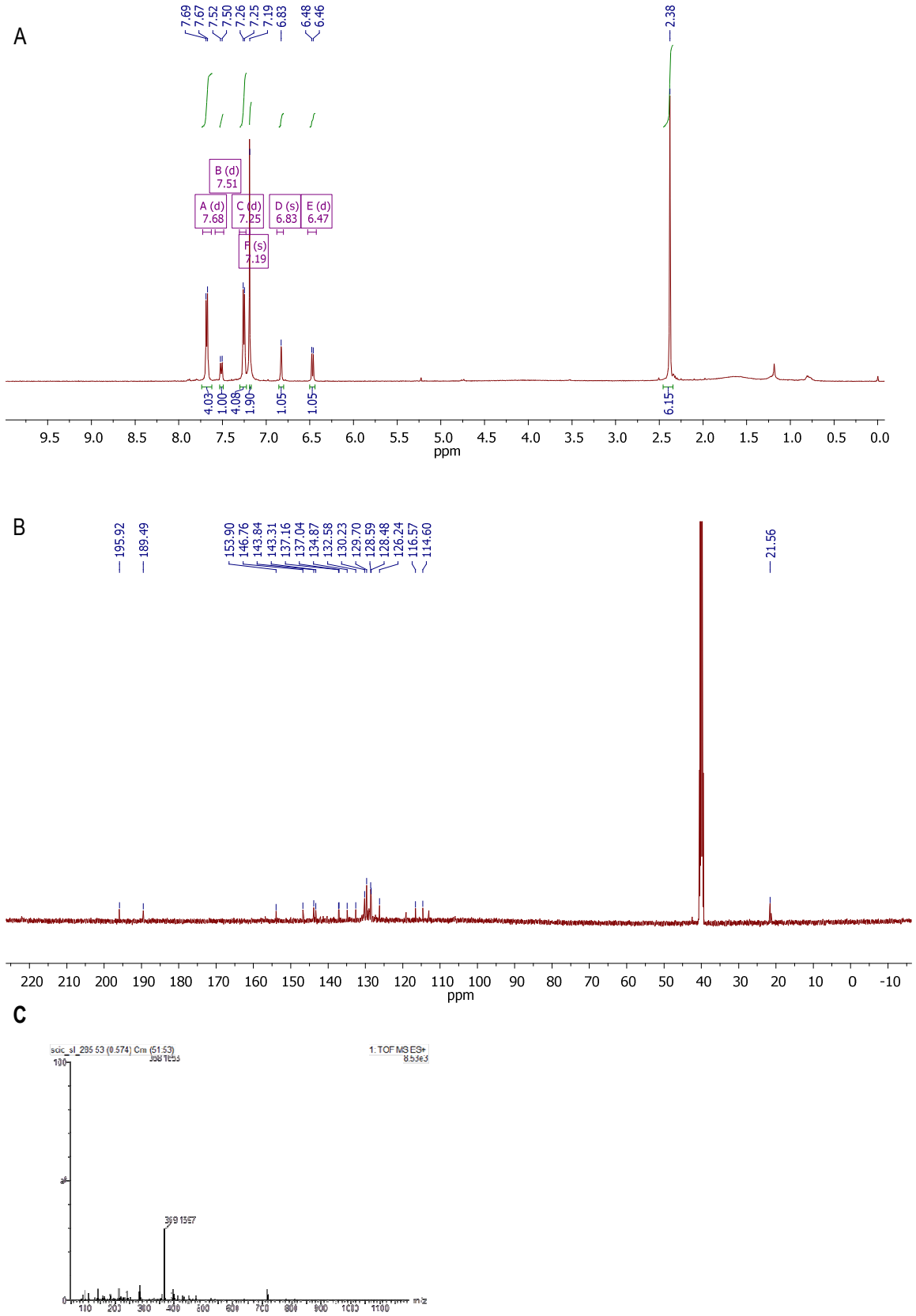


Figure S6. A) ^{1}H NMR ($CDCl_3$) spectra of compound **2**b; B) ^{13}C NMR (DMSO-d_6) spectra of compound **2b**; C) HRMS spectra of compound **2b**

Compound 2c

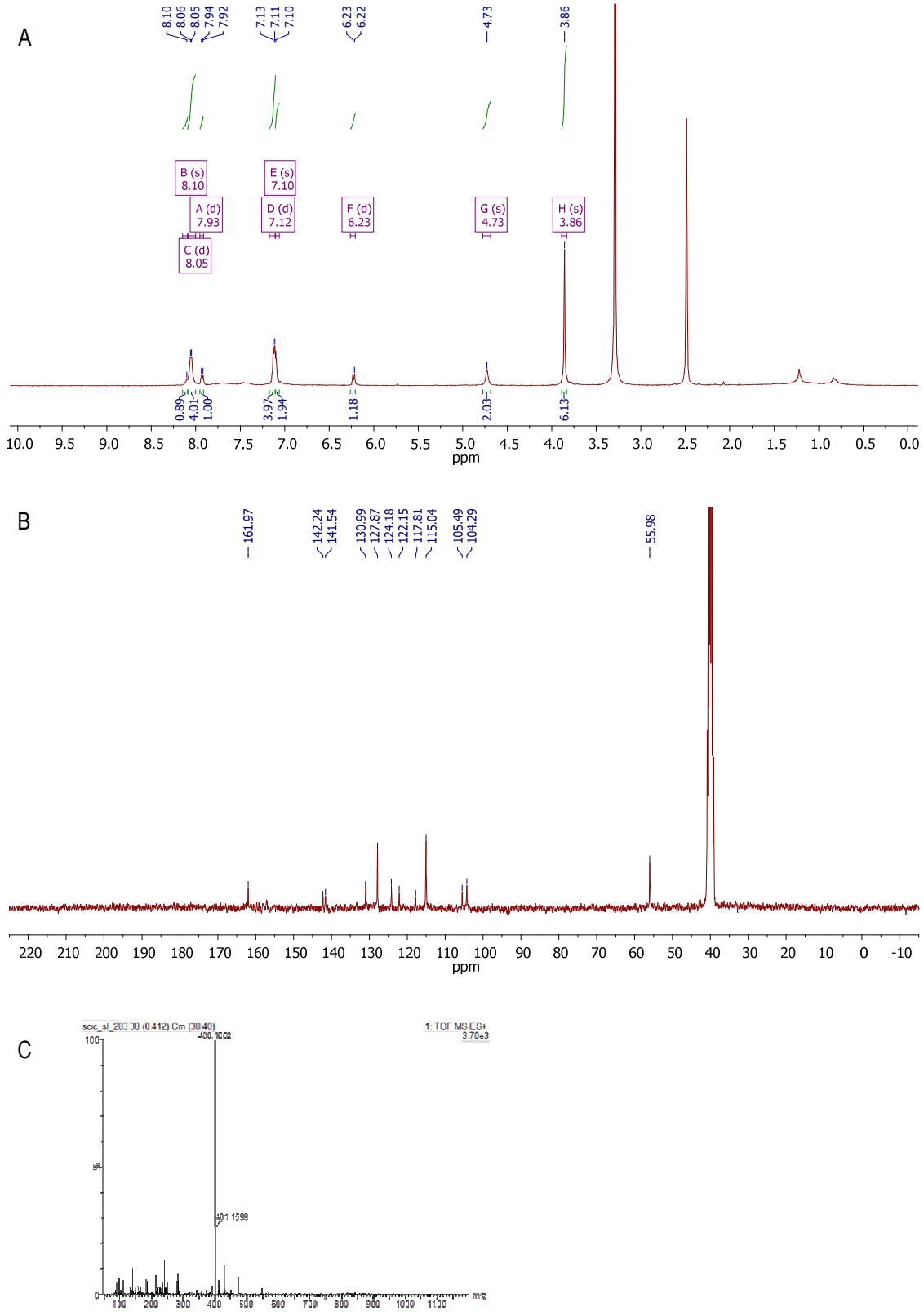


Figure S7. A) ^{1}H NMR (DMSO-d_6) spectra of compound **2c**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **2c**; C) HRMS spectra of compound **2c**

Compound 2d

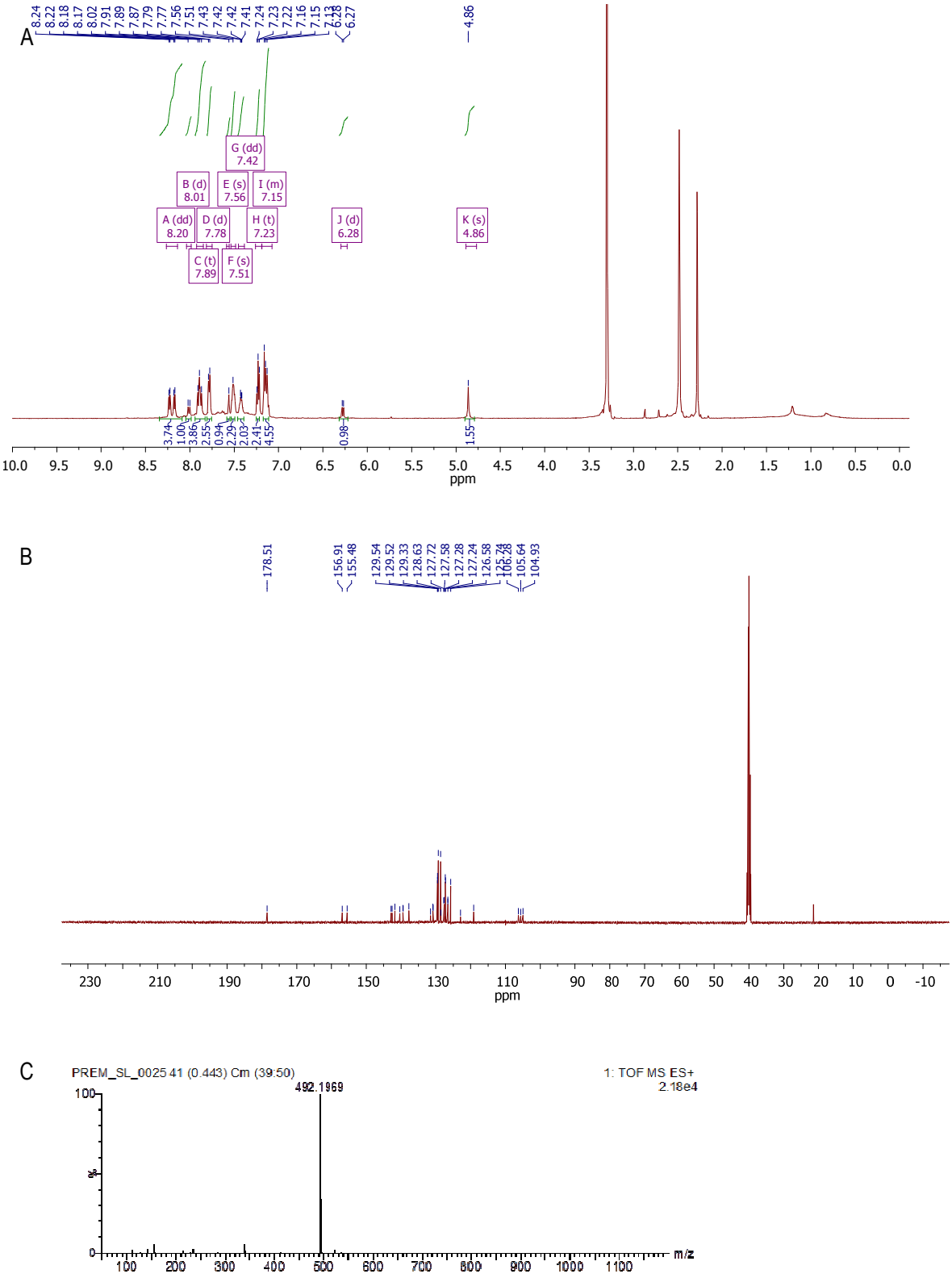


Figure S8. A) ^{1}H NMR (DMSO-d_6) spectra of compound **2d**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **2d**; C) HRMS spectra of compound **2d**

Model compound 3

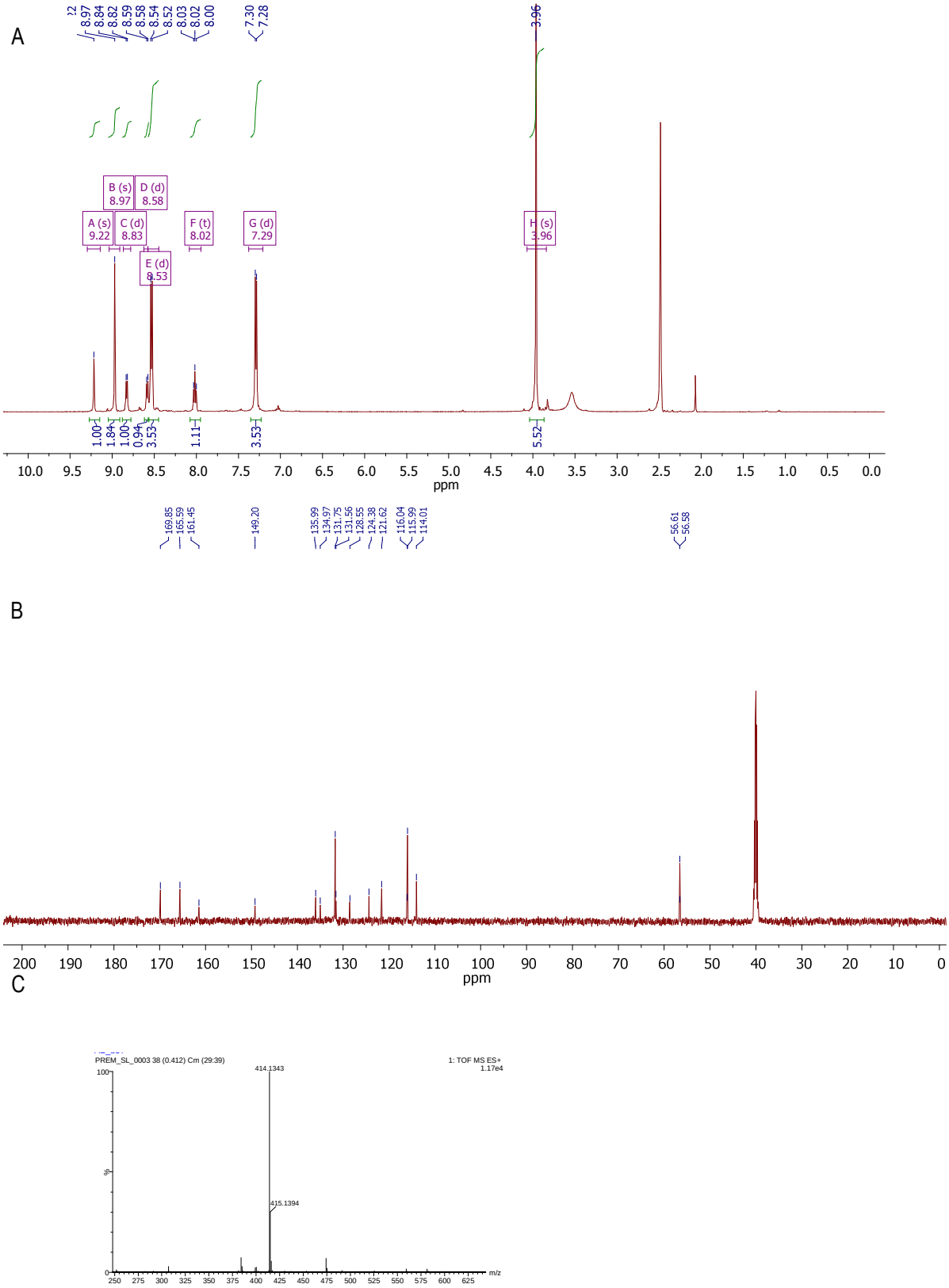


Figure S9. A) ^{1}H NMR (DMSO-d_6) spectra of compound **1e**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **1e**; C) HRMS spectra of compound **1e**

Model compound 4

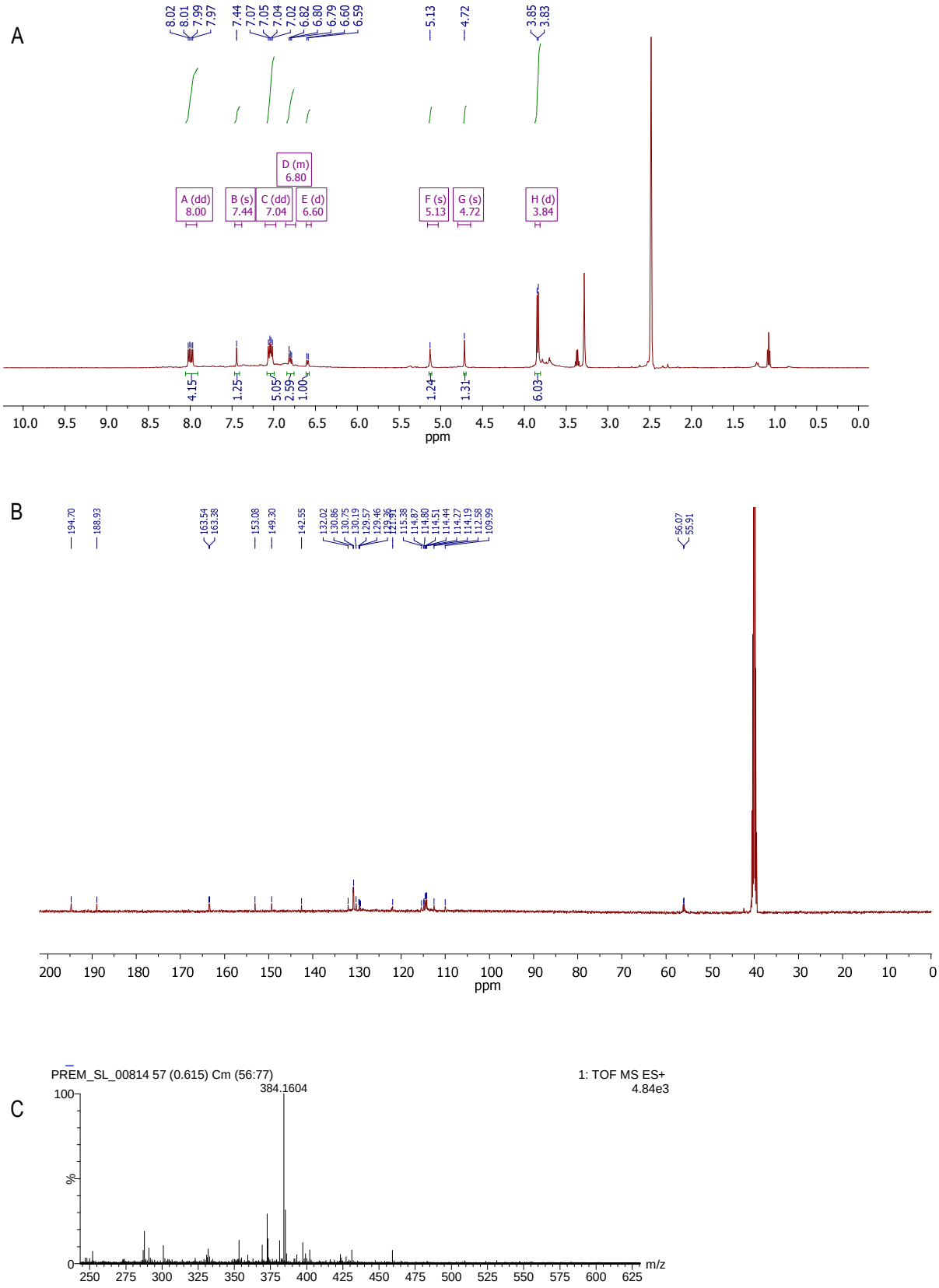


Figure S10. A) ^{1}H NMR (DMSO-d_6) spectra of compound **2e**; B) ^{13}C NMR (DMSO-d_6) spectra of compound **2e**; C) HRMS spectra of compound **2e**

Reaction product of 2a with NO/O_2

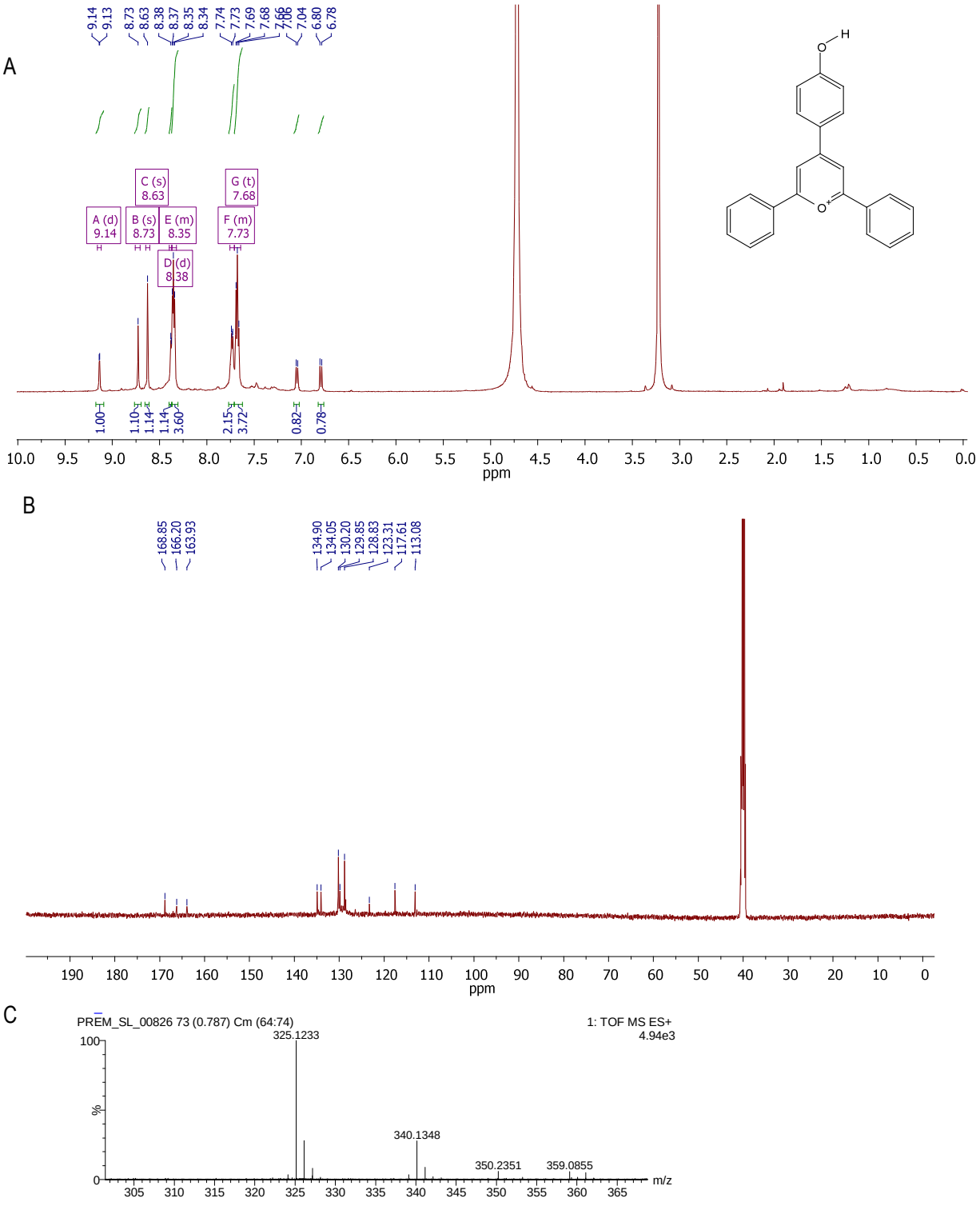
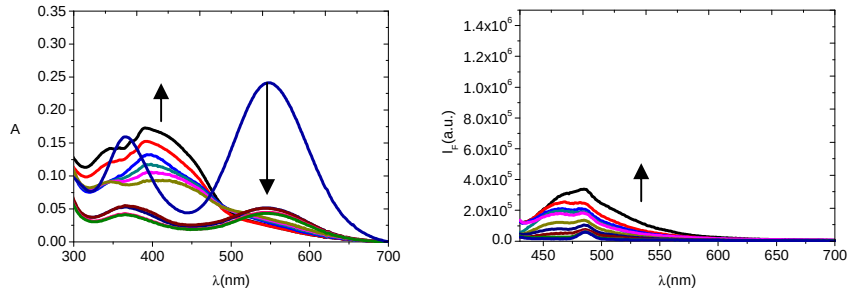


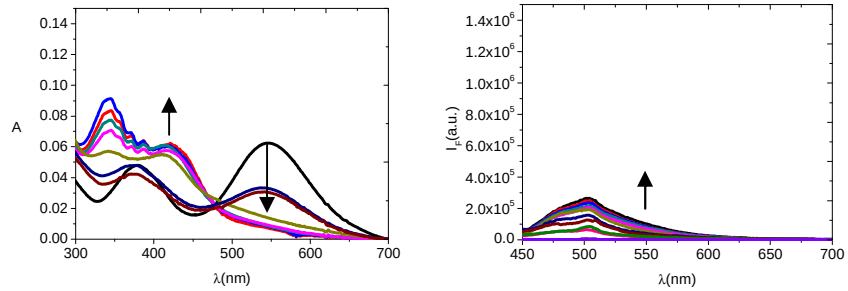
Figure S11. A) ^{1}H NMR (CD_3OD) spectra of reaction product of compound **2a** with NO/O_2; B) ^{13}C NMR (CD_3OD) spectra of reaction product of compound **2a** with NO/O_2, C) HRMS spectra of reaction product of compound **2a** with NO/O_2.

Absorption and fluorescence studies

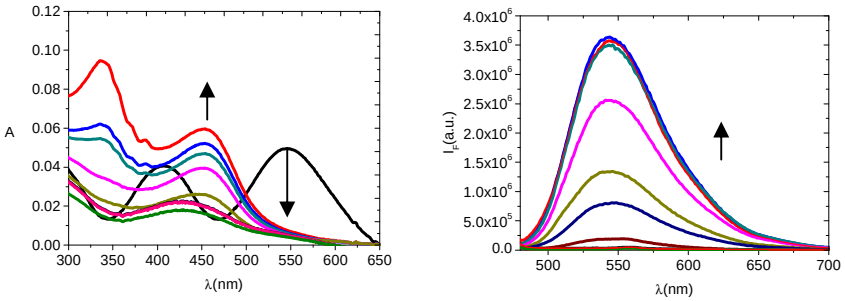
Compound 2a



Compound 2b



Compound 2c



Compound 2d

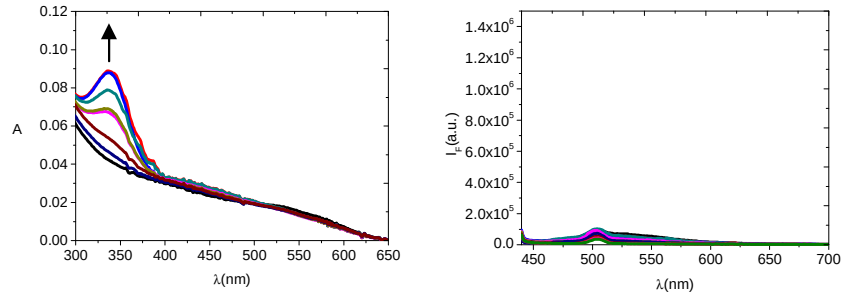


Figure S12 Spectroscopic studies of NO sensing using compounds **2a-d**. First column: Absorption spectra of compound **2a-d** (10µM) in aerated water at pH 7.2 in the presence of increasing amounts of NO (from 0,1µM to 1000µM). Second column: Emission spectra of compound **2a-d** (10µM) in aerated water at pH 7.2 in the presence of increasing amounts of NO (from 0,1µM to 1000µM).

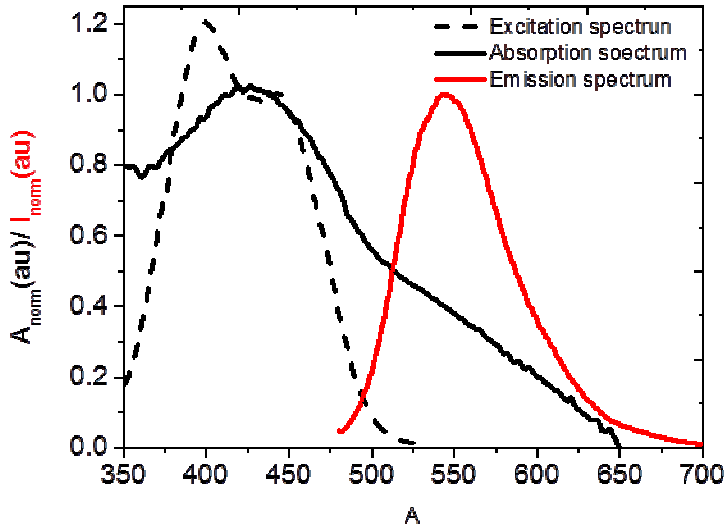
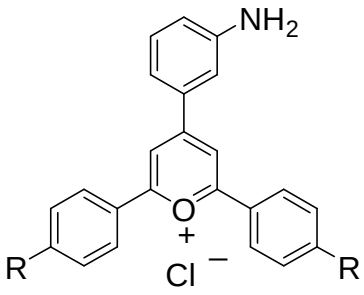


Figure S13 Absorption, excitation and emission spectra of a solution of compound **2c** (10µM) in aerated water at pH 7.2 after reaction with excess of NO (1 mM).

Studies with model compound



4 R = OCH_3

Model compound **4**

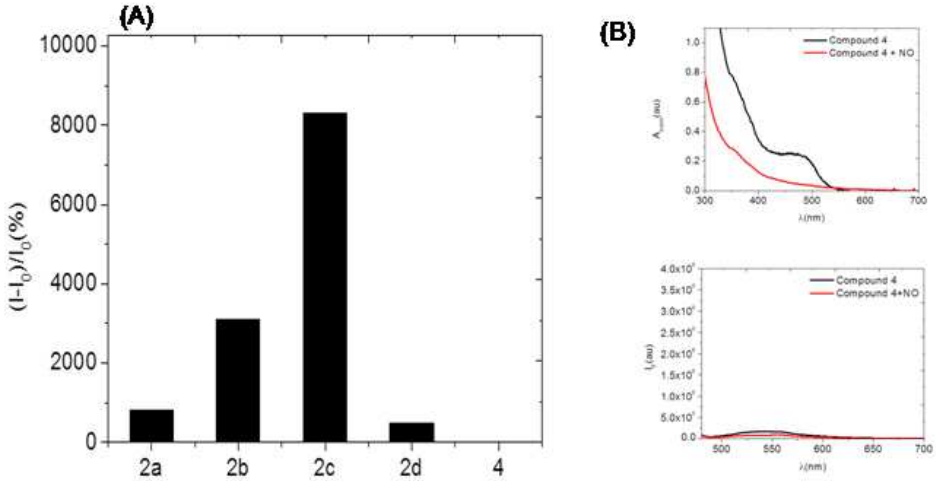


Figure S14 A) Fluorescence enhancement of compounds **2a-e** (10µM) after reaction with 50 equivalents of NO in water (pH 7.2). **B)** On the top: absorption spectra of compound **4** in aerated water at pH 7.2, before and after of adding NO (50 equiv.). On the bottom: emission spectra of compound **4** in aerated water at pH 7.2 before and after of adding NO (50 equiv.).

Studies with dehydroascorbic acid

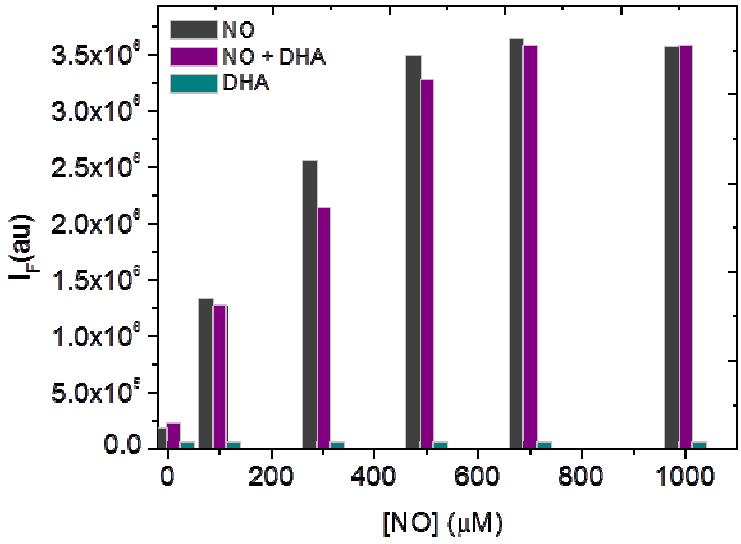


Figure S15. Emission intensity at λ = 550 nm of a solution of compound **2c** (10μM) in aereated water at pH 7.2 in the presence 300 μM of DHA and increasing amounts of NO. Excitation at λ = 470 nm.

X-ray structures

*Chrystallographic data for compound **1a***

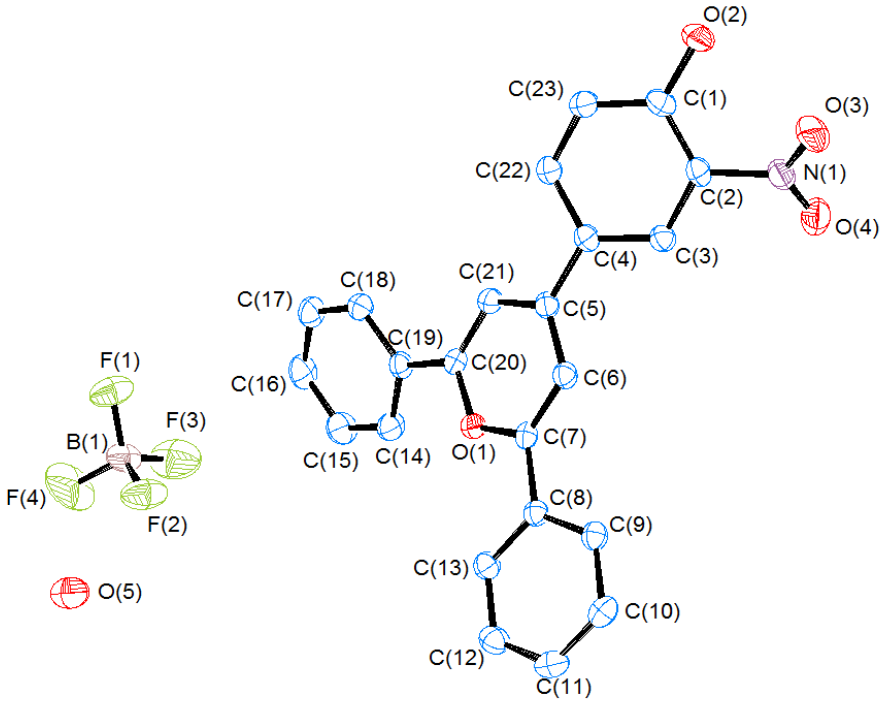


Figure S16. X-ray structure for compound **1a**

Table S1 Crystal data and structure refinement for compound **1a**

Identification code	str1326
Empirical formula	$C_{23}H_{18}BF_4NO_5$
Formula weight	475.19
Temperature/K	298
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	7.16287(15)
b/Å	14.4364(2)
c/Å	20.3056(4)
α/°	90
β/°	96.7873(18)
γ/°	90
Volume/Å^3	2085.01(7)
Z	4
ρ_{calc}mg/mm^3	1.514
m/mm^{-1}	1.114
F(000)	976.0
Crystal size/mm^3	0.21 × 0.059 × 0.041
Radiation	Cu Kα (λ = 1.5418)
2Θ range for data collection	7.532 to 144.272°
Index ranges	$-8 \leq h \leq 8$, $-17 \leq k \leq 17$, $-24 \leq l \leq 25$
Reflections collected	20025
Independent reflections	4053[R(int) = 0.0400]
Data/restraints/parameters	4053/0/306
Goodness-of-fit on F^2	1.054
Final R indexes [I>=2σ (I)]	R_1 = 0.0528, wR_2 = 0.1360
Final R indexes [all data]	R_1 = 0.0648, wR_2 = 0.1429
Largest diff. peak/hole / e Å^{-3}	0.51/-0.47

*Chrystallographic data for compound **2a***

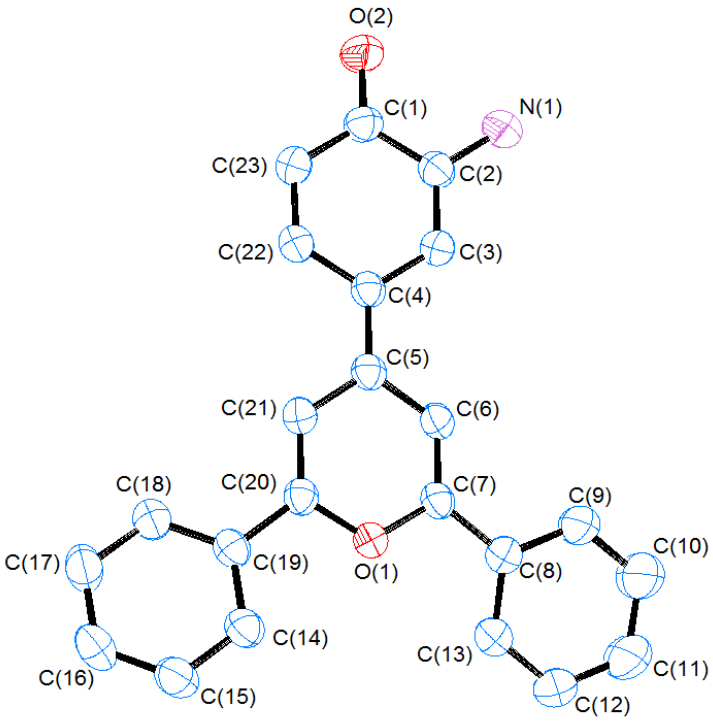


Figure S17. X-ray structure for compound **2a**

Table S2 Crystal data and structure refinement for compound **2a**

Identification code	str1331
Empirical formula	$C_{23}H_{17}NO_2$
Formula weight	339.38
Temperature/K	298
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	8.4285(3)
b/Å	19.3666(4)
c/Å	11.1086(4)
α/°	90
β/°	110.364(4)
γ/°	90
Volume/Å^3	1699.93(11)
Z	4
ρ_{calc}mg/mm^3	1.326
m/mm^{-1}	0.673
F(000)	712.0
Crystal size/mm^3	0.269 × 0.037 × 0.023
Radiation	Cu Kα (λ = 1.5418)
2Θ range for data collection	9.132 to 146.652°
Index ranges	$-9 \leq h \leq 10$, $-24 \leq k \leq 23$, $-13 \leq l \leq 13$
Reflections collected	16164
Independent reflections	3362[R(int) = 0.0325]
Data/restraints/parameters	3362/0/243
Goodness-of-fit on F^2	1.044
Final R indexes [I>=2σ (I)]	R_1 = 0.0562, wR_2 = 0.1532
Final R indexes [all data]	R_1 = 0.0730, wR_2 = 0.1682
Largest diff. peak/hole / e Å^{-3}	0.37/-0.32

*Chrystallographic data for reaction product of **2a** with NO/O_2*

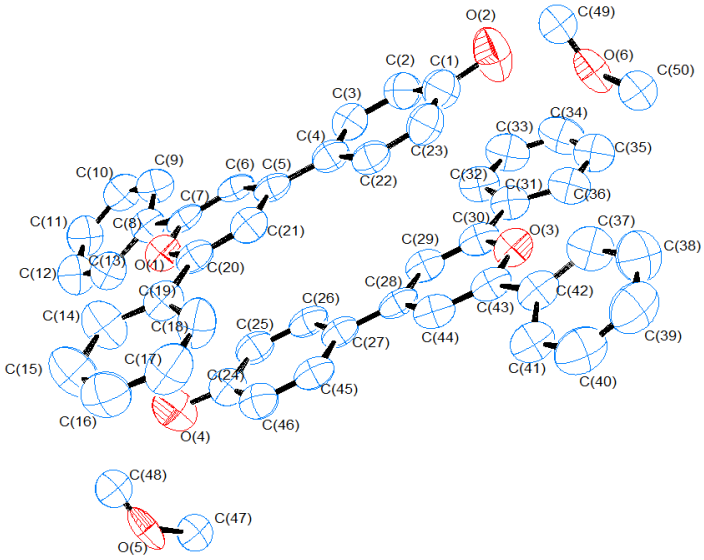


Figure S18. X-ray structure for unit cell of reaction product of compound **2a** with NO/O_2

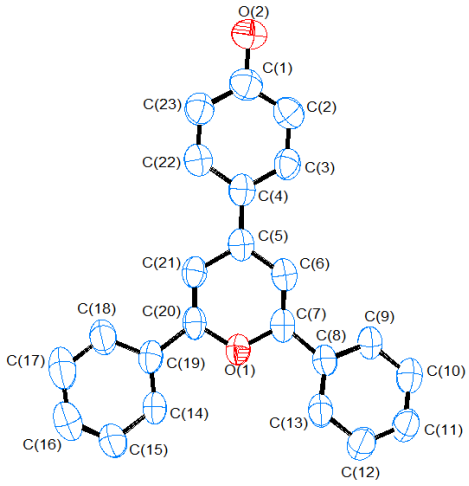


Figure S19. X-ray structure for reaction product of compound **2a** with NO/O_2

Table S3 Crystal data and structure refinement for reaction product of compound **2a** with NO/O_2

Identification code	str1386
Empirical formula	$C_{23.79674}H_{17.86715}O_{2.5}$
Formula weight	343.81
Temperature/K	150.05(10)
Crystal system	orthorhombic
Space group	$P2_12_12$
a/Å	26.9819(14)
b/Å	19.5048(6)
c/Å	7.1392(3)
α/°	90
β/°	90
γ/°	90
Volume/$Å^3$	3757.2(3)
Z	8
$\rho_{calc}mg/mm^3$	1.216
m/mm^{-1}	0.620
F(000)	1445.0
Crystal size/mm^3	0.2817 × 0.0459 × 0.0339
Radiation	Cu Kα (λ = 1.5418)
2Θ range for data collection	6.56 to 146.56°
Index ranges	$-32 \leq h \leq 33$, $-24 \leq k \leq 23$, $-6 \leq l \leq 8$
Reflections collected	20164
Independent reflections	6941[R(int) = 0.0402]
Data/restraints/parameters	6941/2/477
Goodness-of-fit on F^2	1.021
Final R indexes [I>=2σ (I)]	$R_1 = 0.0806$, $wR_2 = 0.2359$
Final R indexes [all data]	$R_1 = 0.1032$, $wR_2 = 0.2602$
Largest diff. peak/hole / e $Å^{-3}$	0.69/-0.23
Flack parameter	0.5(2)

*SQUEEZE has ben used to eliminate some peaks of high electron density that could not be modeled either as a solvent or as an anion . This procedure reduce R_1 value.

Biological studies

Materials

Cell culture media and supplements were obtained from Invitrogen. Bacterial lipopolysaccharide (LPS) and L-nitro-monomethyl-arginine (L-NMMA) were obtained from Sigma. IFN-γ was obtained from R&D Systems Europe (Abingdon, UK).

Methods

Cell culture and treatments: RAW264.7 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. For treatments, cells were incubated in RPMI1640 without phenol red, supplemented with antibiotics. Cells were activated by addition of 10 μg/ml LPS and 100 U/ml IFN-γ.[8] Inhibition of NOS activity was achieved by addition of 300 μM L-NMMA 20 min before stimulation.

Nitrite determination

Accumulation of nitrite in the cell culture supernatant, as an index of iNOS activity, was measured by the method of Griess, as previously described.[9]

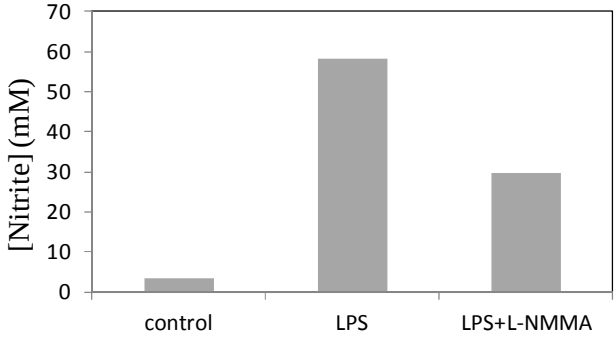


Figure S20. An aliquot of the cell supernatant was used for nitrite determination. Results shown are representative from three independent assays.

Fluorescence microscopy

Cells were cultured in glass bottom dishes (Mattek Corporation) and activated as described above. For fluorescence detection of NO, the **2c** probe dissolved in DMSO was added at 20 μM final concentration. Control cells received an equivalent amount of DMSO (0.1% (v/v)). After 30 min incubation at 37°C, live cells were directly visualized on a Leica DMRE2 confocal microscope. The probe was excited at 488 nm with an argon laser and emission between 500 and 600 nm was collected. Sections were taken every 1 μm and total projections are shown.

[8] E. Cernuda-Morollón, F. Rodríguez-Pascual, Klatt, S. Lamas, D. Pérez-Sala , *J. Am. Soc. Nephrol*. **2002**, 13, 2223.

[9] M. Saura, D. Pérez-Sala, F.J. Cañada, S. Lamas , *J. Biol. Chem*. **1996**, 271, 14290