Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

The First Purification and Unequivocal Characterization of the Radical Form of the Carbon-centered Quinone Ketoxy Radical Adduct

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Materials and Methods

Materials

2,5-dichloro-1,4-benzoquinone (DCBQ), *t*-butylhydroperoxide (*t*-BuOOH) and DMPO (5,5-dimethyl-1-pyrroline *N*-oxide) were purchased from Sigma-Aldrich. HPLC-grade acetonitrile was obtained from J. & K. Chemical Ltd. BMPO was synthesized according to published method [1].

HPLC/ESI-Q-TOF-MS Study

The final products of the reaction BMPO/*t*-BuOOH/DCBQ were analyzed by HPLC coupled with ESI-Q-TOF-MS. HPLC apparatus was equipped with a model 2996 photodiode array detector (Waters; 2695XE). The separation column was Eclipse XDB-C18 (15 cm × 4.6 mm, 5 μm) from Agilent. Optimum separation was achieved with a binary mobile phase with a flow rate at 1.0 mL/min. Capillary and sample cone voltages were 2.5 kV and 30 V; Source and desolvation temperatures were 80°C and 200°C. Nitrogen was used as the drying gas. The collision gas was argon at the pressure of 5.0×10^{-5} Torr (1 Torr = 133.322 Pa), and the collision energy was 10 V. Both the high and low resolution for mass filter was set at 5.0V. The pressure in the TOF cell was lower than 3.0×10^{-7} Torr. Full-scan spectra were recorded in profile mode. The range between m/z 50 and 450 was recorded at a resolution of 5,000 (FWHM), and the accumulation time was 1 s per spectrum. For MS/MS studies, the quadrupole was used to select the parent ions, which were subsequently fragmented in a hexapole collision cell by using argon as the collision gas and an appropriate collision energy (typically 10–20 eV). Data acquisition and analysis were carried out by using MassLynx software (Waters; Version 4.0).

High resolution FTICR-MS Study

The BMPO adducts were further detected on a 7.0 tesla FTICR (Bruker APEX IV). Reactions were carried out in Chelex-treated phosphate buffer (100 mM, pH 7.4). BMPO, 50 mM; DCBQ, 1 mM; *t*-BuOOH, 100 mM; CH_3CN, 70%. FTICR was calibrated using Tuning mix. The range is between *m/z* 100 and 3000. End Plate electrode voltage for ESI: 2300 V, Capillary Entrance voltage for ESI: 3000 V, Skimmer1 voltage for ESI: -30 V; Dry gas temperature 200°C; Dry gas Flow rate 12 L/min; Neb gas Flow rate 6 L/min. Data acquisition and analysis were carried out by using Bruker Compass DataAnalysis software.

Semi-preparative HPLC Methods

The reaction products were isolated by an Agilent 1200 HPLC apparatus (Agilent Tech. Corp) equipped with a UV detector. Reaction solution (500 μL) was injected into a semi-preparative HPLC column (15 cm × 10.0 mm, 3 μm, SUPELCOSIL C-18, Sigma). Samples were eluted at a flow rate of 3 mL/min with a deionized water–CH_3CN gradient [CH_3CN, 10–15% (linear, 10 min), 15–50% (linear, 10 min)]. The fractions were monitored at 278 nm and the fraction with retention time 2.62 min was collected manually with heart cutting.

ESR studies

DCBQ and BMPO were dissolved in CH_3CN. Reactions were carried out in Chelex-treated phosphate buffer (100 mM, pH 7.4). The solutions were recorded 1 min after the interactions between *t*-BuOOH and DCBQ at room temperature under normal room-lighting conditions on a Bruker ER 200 D-SRC spectrometer operating at 9.54 GHz and a cavity equipped with a Bruker Aquax liquid sample cell. Typical spectrometer parameters were as follows: scan range, 100 G; field set, 3405 G; time constant, 200 ms; scan time, 100 s; modulation amplitude, 0.25 G; modulation frequency, 100 kHz; receiver gain, 1.25×10^5; and microwave power, 20 mW. The hyperfine splitting constants were measured by using the simulation software WinSim (version 0.96) (NIEHS) [2].

References

1. H. T. Zhao, J. Joseph, H. Zhang, H. Karoui and B. Kalyanaraman, *Free Radic. Biol. Med.*, 2001, **31**, 599-606.
2. D. R. Duling, *J. Magn. Reson. B*, 1994, **104**, 105-110.

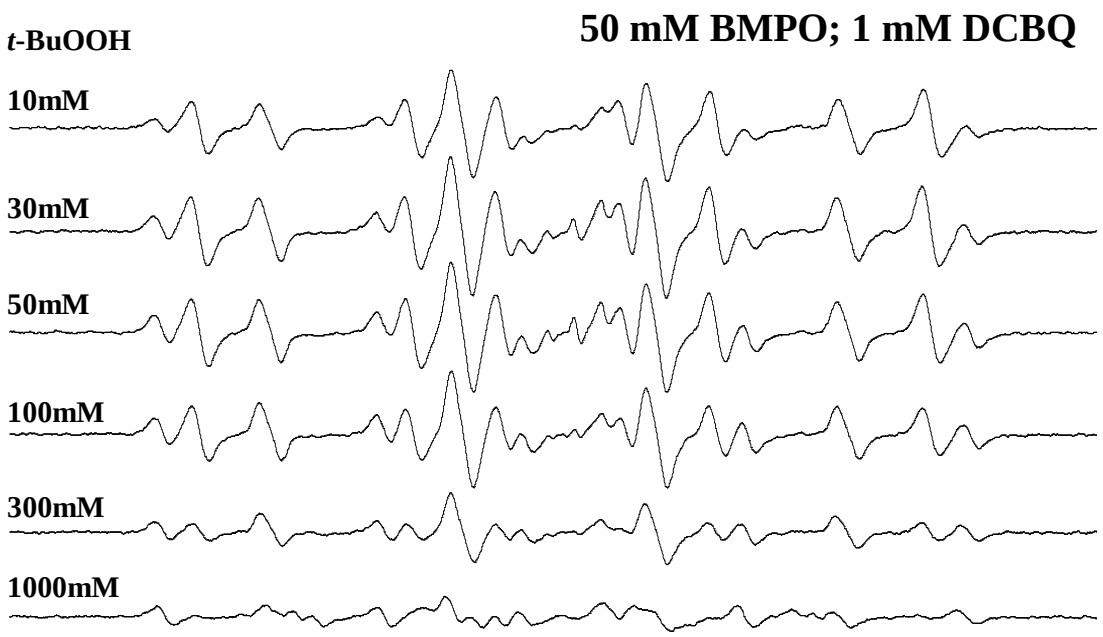

Fig. S1: The formation of BMPO/•CBQ-OH depends on t-BuOOH concentrations. Reactions were carried out at room temperature in Chelex-treated phosphate buffer (100 mM, pH 7.4). BMPO, 50 mM; DCBQ, 1 mM; *t*-BuOOH, 1 mM, 3 mM, 10 mM, 30 mM, 50 mM, 100 mM, 300 mM, 1000 mM; 1% CH_3CN.

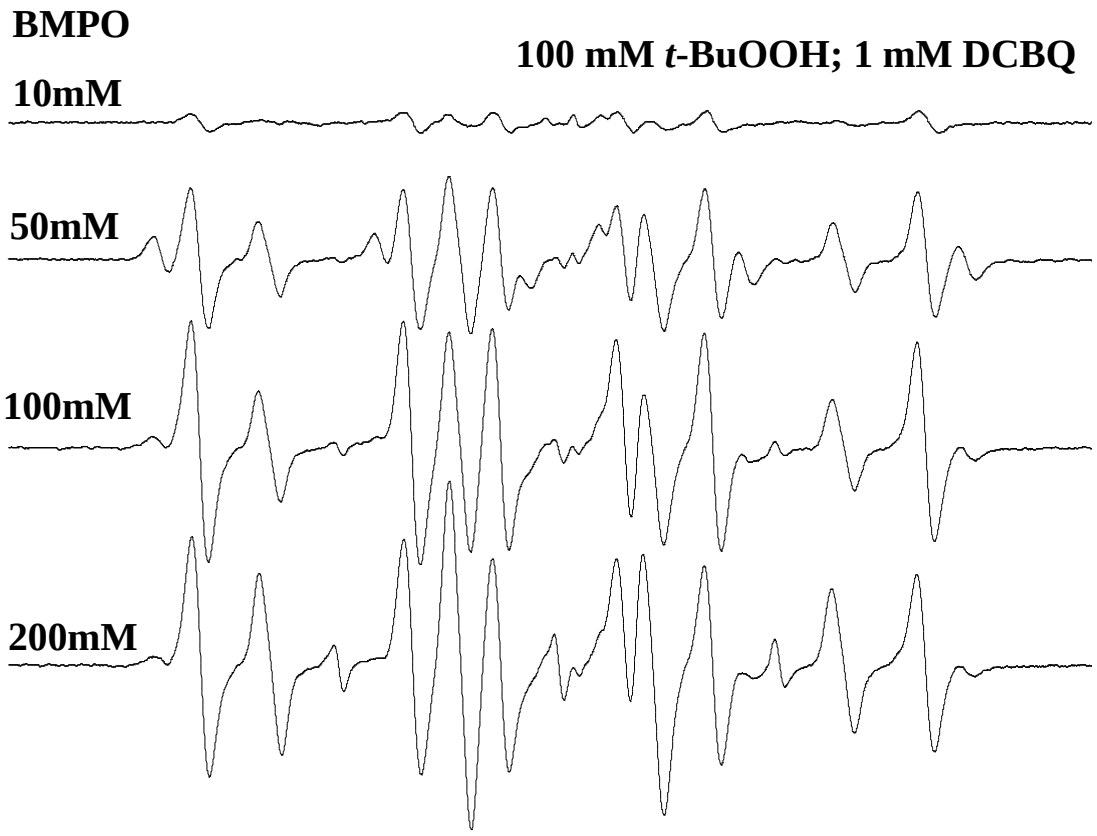

Fig. S2: The formation of BMPO/•CBQ-OH depends on BMPO concentrations. Reactions were carried out at room temperature in Chelex-treated phosphate buffer (100 mM, pH 7.4). BMPO, 10 mM, 50 mM, 100 mM, 200 mM; DCBQ, 1 mM; *t*-BuOOH, 100 mM; 1% CH_3CN.

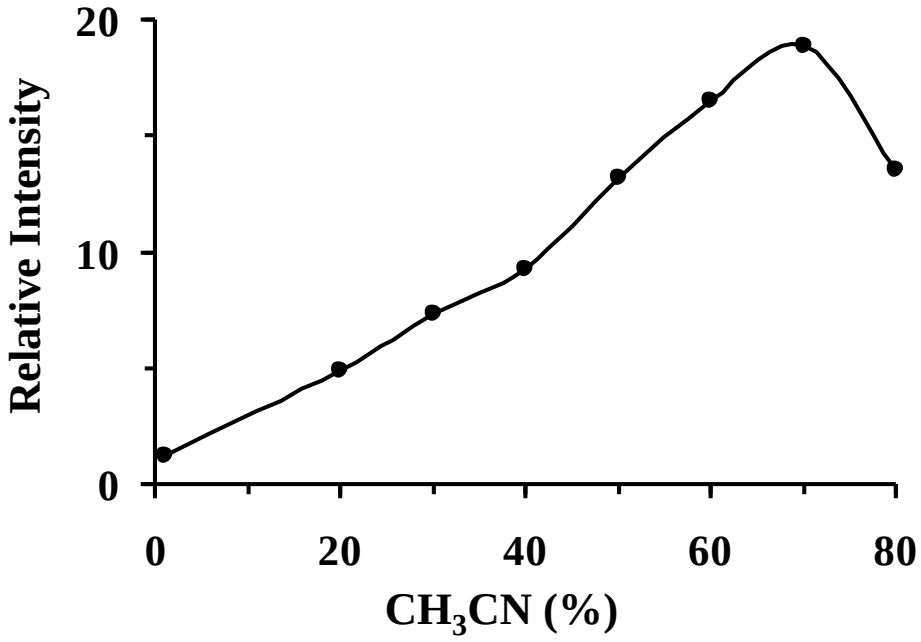

Fig. S3: The formation of BMPO/•CBQ-OH depends on CH_3CN concentrations. Reactions were carried out at room temperature in Chelex-treated phosphate buffer (100 mM, pH 7.4). BMPO, 50 mM; DCBQ, 1 mM; *t*-BuOOH, 100 mM; CH_3CN, 1%, 20%, 30%, 40%, 50%, 60%, 70%, 80%. The BMPO/•CBQ-OH intensity is calculated by the height of the first peak on the left of ESR spectra.

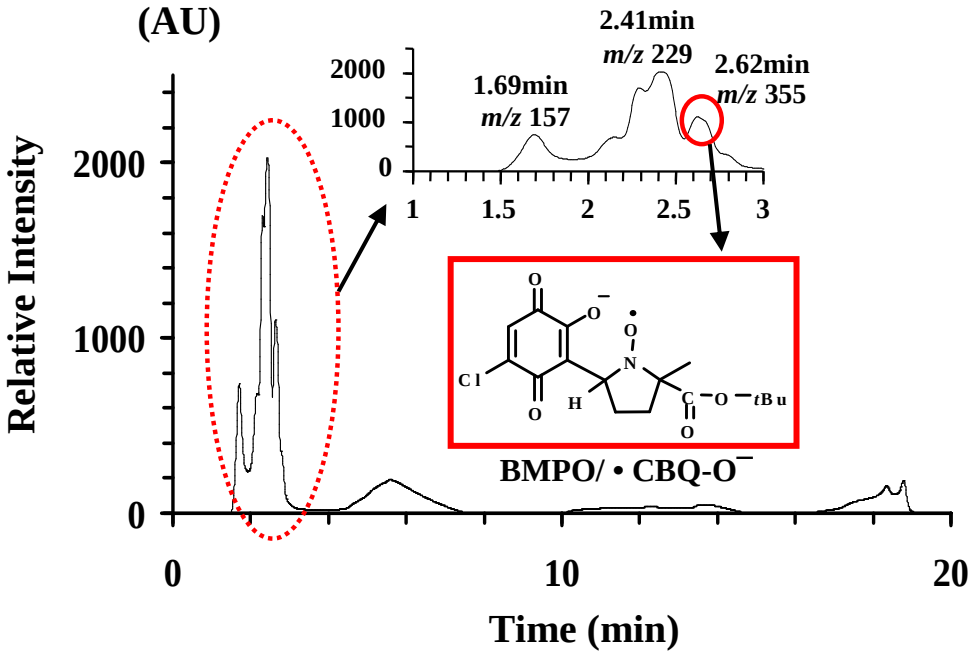

Fig. S4: Semi-preparative HPLC separation of radical form of BMPO/•CBQ-OH adducts. Reactions were carried out in Chelex-treated phosphate buffer (100 mM, pH 7.4). BMPO, 50 mM; DCBQ, 1 mM; *t*-BuOOH, 100 mM ; CH_3CN, 70%. Reaction solution (500 µL) was injected directly into a semi-preparative HPLC column (15 cm × 10.0 mm, 3 µm, SUPELCOSIL C-18, Sigma). Samples were eluted at a flow rate of 3 mL/min with a deionized water-CH_3CN gradient [CH_3CN, 10–15% (linear, 10 min), 15–50% (linear, 10 min)].