Benzothiazole Sulfinate: a Water Soluble and Slow Releasing Sulfur Dioxide Donor

Supporting Information

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Materials and methods

Sodium bisulfite ($NaHSO_3$) was purchased from Aldrich (Milwaukee, WI). All buffers were prepared using standard methods with nano-pure DI water. pH 4 and 5 were 0.1 M acetic acid/acetate buffer, pH 6 and 7.4 were standard 1x PBS buffers. All absorbance data for kinetics measurements were performed using a Thermo Scientific Evolution 300 UV-vis spectrometer. All fluorescence measurements were performed on an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer. HPLC measurements were performed using Thermoscientific HPLC with a Finnigan Surveyor detector equipped with a Thermo RP C18, 100 X 4.6 mm, 5 µM (particle) column. ^1H and ^{13}C NMR spectra were obtained using a Varian 300 (300 MHz for ^1H and 75 MHz for ^{13}C). 1H and 13C NMR chemical shifts are reported in parts per million (ppm).

General procedure for the measurements of BTS decomposition in buffers

Using UV absorbance: solutions of BTS at concentrations ranging from 100-400 µM were prepared in pH 4, 5, 6, and 7.4 buffers. These were incubated at 37 ^oC, in random time increments, the absorbance was measured scanning from 250 nm to 350 nm (Figure S1). The absorbance at 310 nm was then converted to concentration using molar absorptivity of ε = 1826 M^{-1} cm^{-1} and the initial rate of reaction was established

via linear regression. Benzothiazole (BTH) and sulfur dioxide have no absorbance at 310 nm in micromolar concentrations.

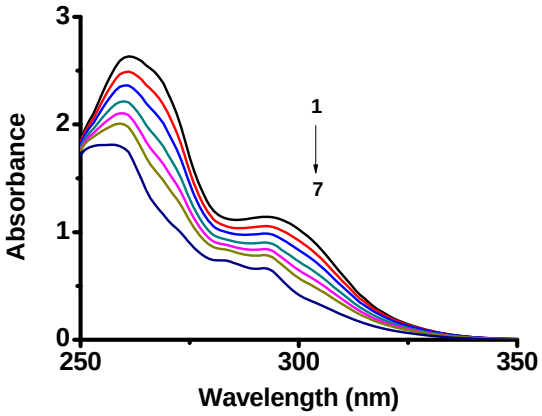
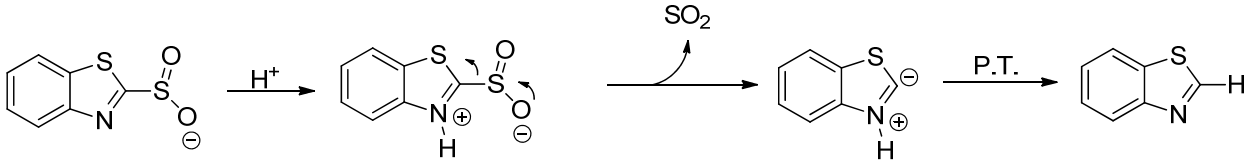


Figure S1. Representative absorbance spectrum of BTS scanning from 250 nm to 350 nm in pH 5 buffer from 1 to 120 minutes (1-7).

Using HPLC: solutions of BTS at concentrations ranging from 100-400 µM were prepared in pH 4, 5, 6, and 7.4 buffers. These were incubated at 37 ^{o}C and in random time increments 20 µL of the solutions were injected onto the HPLC. Gradient: 30% methanol in water to 95% methanol in water over 15 minutes was used monitoring 255 nm absorbance for 10 minutes. Flow rate was 1.0 mL/min with 20 µL injection volume. BTS Rf= 1.44 mins. BTH Rf= 6.99 mins.



Scheme S1. Proposed SO_2 release mechanism from BTS

To support this proposed mechanism, HCl-promoted decomposition of BTS was carried out in D_2O. Briefly, BTS (60 mg) was dissolved in D_2O (10 mL). To this solution was added 3 drops of concentrated HCl. The reaction was stirred at rt for 12 hours. The only isolated product was found to be C2-deuterium quenched benzothiazole.

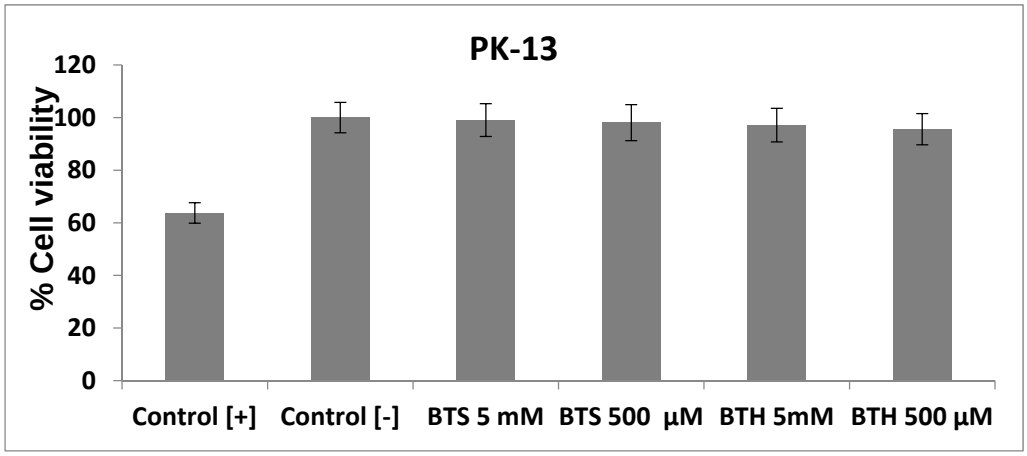
General procedure for fluorescence measurements

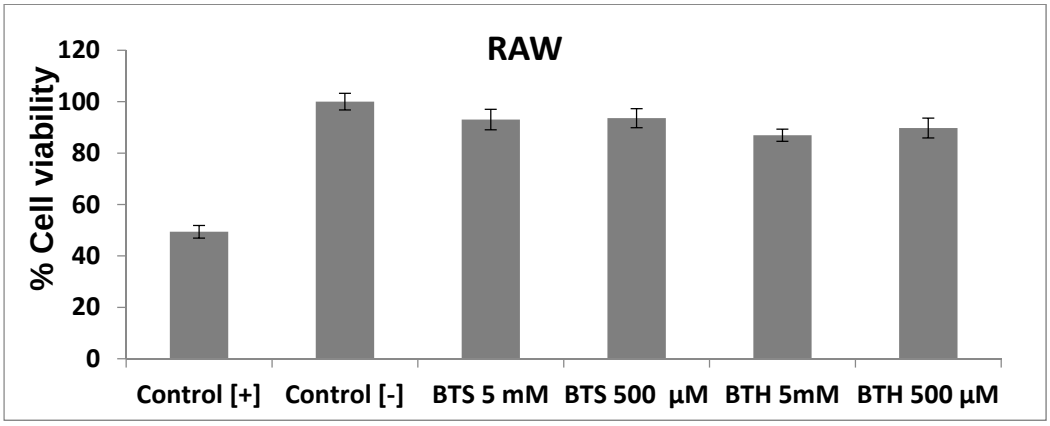
A previously reported ratiometric SO_2 probe, mito-ratio-SO_2, was synthesized according to literature procedures.[1] 5 mM BTS and $NaHSO_3$ stock solutions were freshly

prepared in 4 mL nano-pure DI water. 800 µL of the solution was diluted to 4 mL in pH 4, 5, 6, and 7.4 buffers to make a 1 mM solution. These were sealed and incubated for 1 hour at 37 ^{o}C. 800 µL of each solution was then diluted to 4 mL in pH 7.4 buffer to make 200 µM solutions. To each 4 µL of a 2 mM Mito-ratio-SO_2 probe stock solution in DMSO was added to give a 2 µM solution of the sensor. The mixture was incubated for 30 mins at 37 ^{o}C. A blank was also prepared using pH 7.4 buffer. Fluorescence emission was measured (excitation= 405 nm, scanning 420-700 nm, 800 V) and the ratiometric intensity of 481 nm over 651 nm was calculated.

Cytotoxicity studies of BTS and BTH

90% confluent PK-13, H9C2, and RAW cells were incubated with 0.5 mM or 5 mM of either BTS or BTH at 37 ^{o}C for 20 hours in triplicates. Positive controls were treated with 20% H_2O_2 and negative controls were treated with buffer only. Cells were treated with CCK-8 assay (Dojindo) for one hour to assess cell viability on a Tecan Infinity M1000 plate reader.





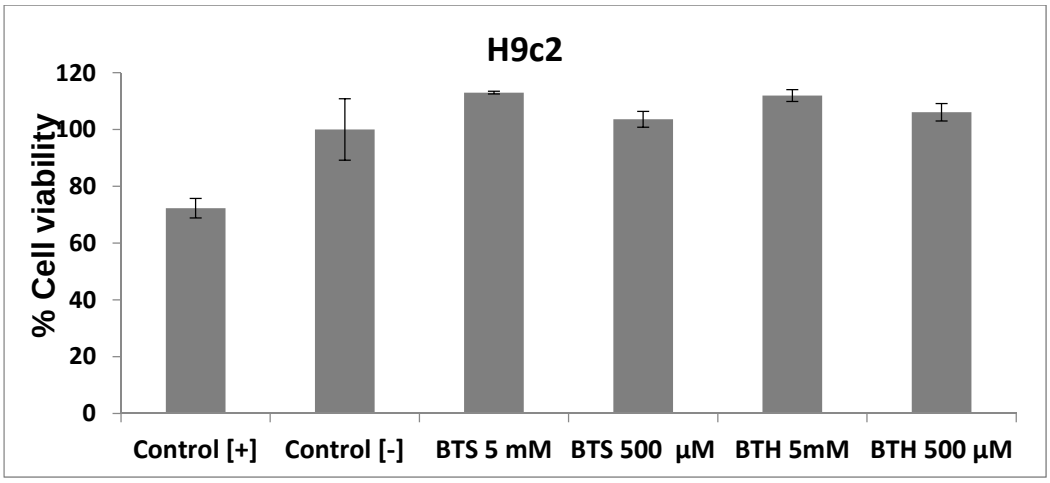


Figure S2. Cell viability of PK-13, RAW, and H9c2 cells incubated with 5 mM and 0.5 mM BTH and BTS after 20-hour incubation.

Preparation of isolated rat thoracic aorta rings

Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (Documentation 55, 2001) and the Animal Care Committee of Shanxi University.

Male Wistar rats weighing about 220–250 g were obtained from Heibei Medical University (Shijiazhuang, China). Rats were sacrificed by anesthetic overdose (intraperitoneal injection of pentobarbital sodium). The thoracic aorta rings were prepared carefully so that the endothelial cells were not damaged. The thoracic aorta was removed immediately and dissected fat and connective tissues, then cut into rings about 3 mm long. The rings were placed in a bath of Krebs solution at pH 7.4, 37 ^{o}C under a resting optimal tension of 1.5 g while 95% O_2 and 5% CO_2 were bubbled through the solution. Tensions were recorded with a MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China) during the experiment. The aortic rings were allowed to equilibrate for 1 h in bath tubes before the experiment and the Krebs solution was changed every 15 min. The viability of the ring preparation was assessed by contracting vessels with 60 mM KCl before each experiment.

Control experiments using benzothiazole

Using the protocol described in the main text, the effects of benzothiazole on vasorelaxation were also measured. Based on SO_2 release kinetic data, BTS (at 0.25, 0.5, 1, 2, 4 mM) should produce benzothiazole at 1, 2, 4, 8, 16 μM, respectively. These doses of benzothiazole were used in this study. As shown in Figure S3, benzothiazole did not cause any vasorelaxation. The effects were similar to the negative control (with only buffer).

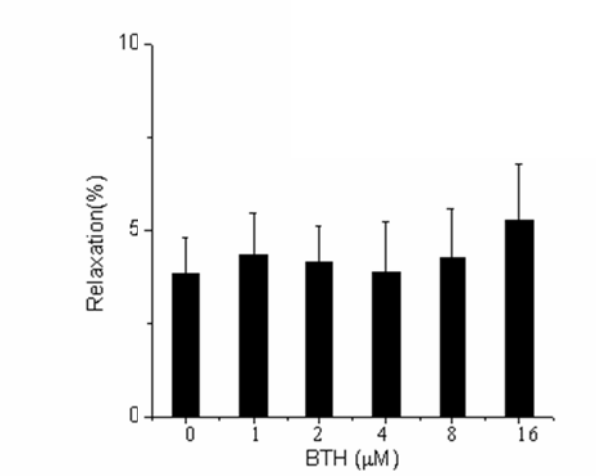
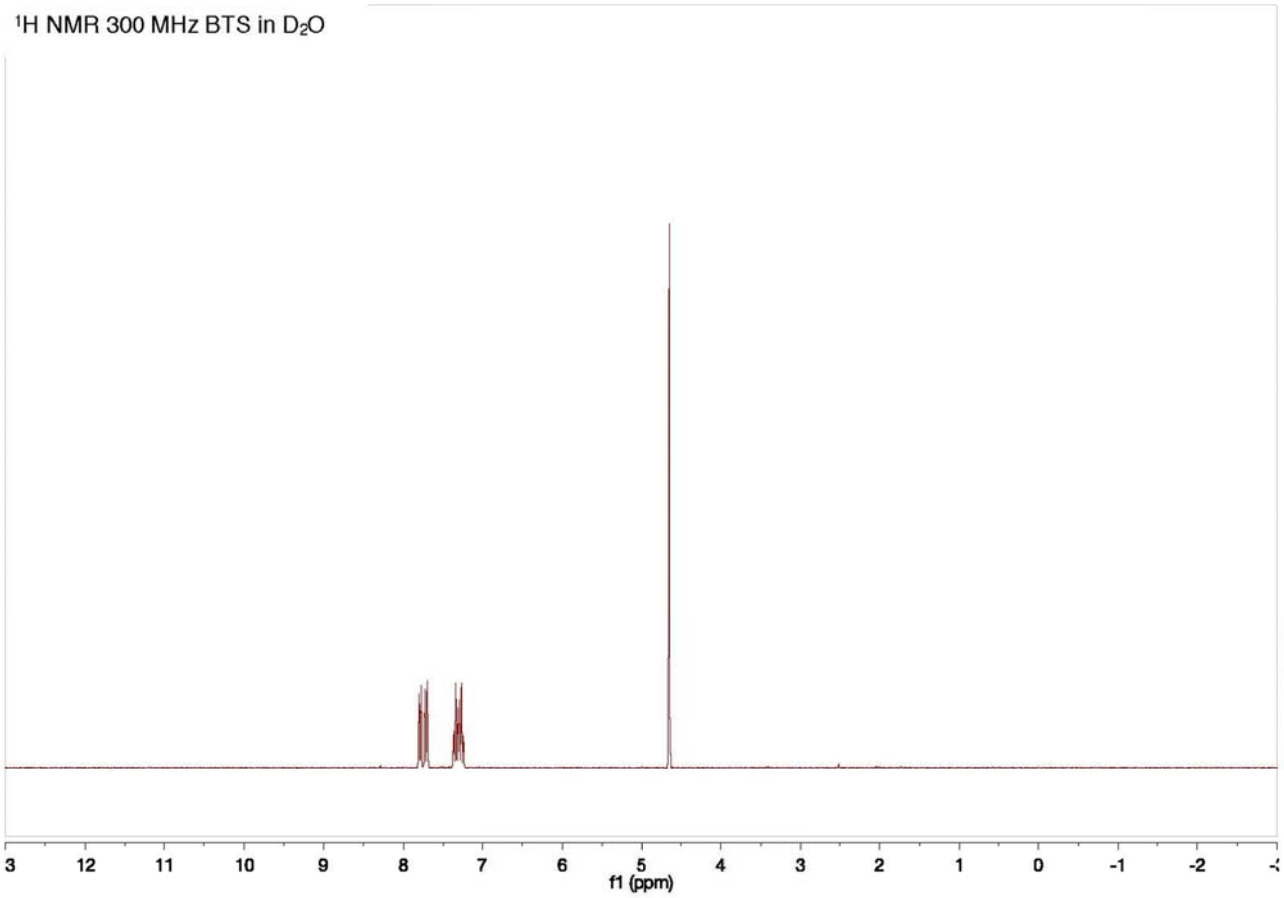


Figure S3, Vasorelaxation of rat aorta by benzothiazole, compared with the control group.

References

1) Xu, W., Teoh, C. L., Peng, J., Su, D., Yuan, L., and Chang, Y.-T. (2015) A mitochondria-targeted ratiometric fluorescent probe to monitor endogenously generated sulfur dioxide derivatives in living cells. *Biomaterials 56*, 1–9.


¹H NMR 300 MHz BTS in D₂O
3
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)

^{13}C BTS NMR in D_2O