Supplementary information 1 - In vitro H_2S gas release

Free (biologically active) sulfide constitutes H_2S in the form of the dissolved undissociated gas and the hydrosulfide anion, HS^-. The dibasic anion S^{2-} has recently been demonstrated to be non-existent in aqueous solutions (May et al., 2018). The assay described in detail below, relies on detection of free H_2S gas that is measured using a commercially available H_2S detector (Z900XP, Environmental sensors, Boca Raton, FL, USA). The default protocol that we have established is as follows:

1) Dissolve compound (ATTM or ATTT) in room temperature phosphate-buffered saline (PBS; pH 7.4) to 10x stock solutions in Eppendorf tubes (1 ml volume). The typical 10x stock concentration is 250 mmol l^{-1} for the thiometallates (1 M total sulfur). For reference, 3 mmol l^{-1} stocks of simple salts e.g. NaHS, generate approximately the same quantity of H_2S under these conditions (Dyson et al., 2017).
2) Vortex for 30-40 seconds.
3) Dilute 1/10 (0.5 into 4.5 ml) rapidly into airtight Falcon tubes (50 ml; Corning Science Mexico, Reynosa, Mexico) containing PBS. The PBS is typically pre-warmed to 37^{o}C but can be adjusted as necessary (e.g. to different temperatures, pH levels, or to contain thiols, other adjuvants or alternative matrices). The liquid and gas (headspace) phases constitute 5 and 45 ml, respectively.
4) Replace the Falcon tube cap, tighten and further seal the lid with Parafilm (Bemis, Neenah, WI, USA).
5) Typically incubate in a water bath for one hour at 37^{o}C.
6) Remove from the water bath and puncture one side of the Falcon tube lid with an orange (25 gauge) needle (Terumo, Egham, Surrey, UK). Puncture the other side of the Falcon tube lid with a blue (23-gauge needle) attached to a 5 ml syringe. Withdraw the 5 ml of headspace gas over 10 seconds.

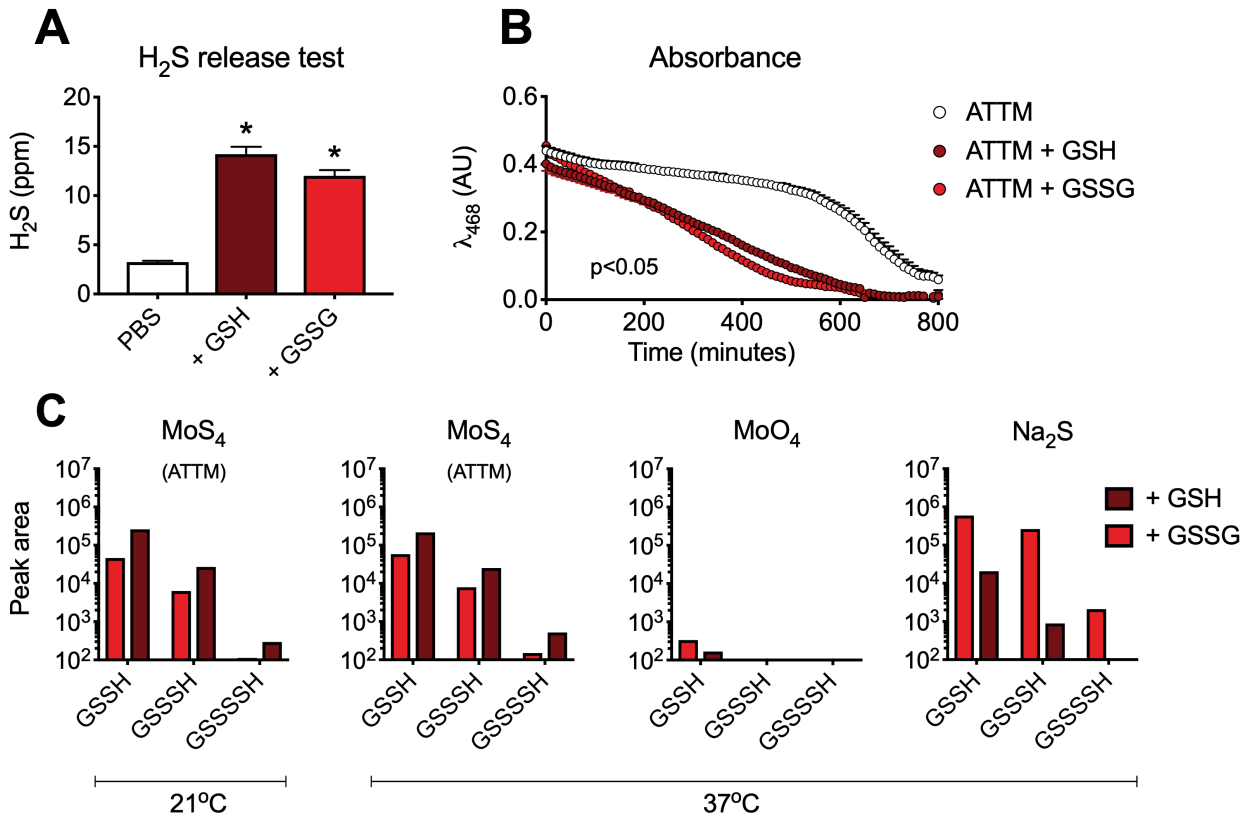
Pass the gas sample through the detector using a 3-way tap (closed to room air) to accommodate the syringe; this is attached to the detector inlet, as shown inset in Fig 1A. Once all of the gas has been drawn from the syringe, *remove* to allow room air to wash out the system. Failure to remove the syringe causes a build-up of H_2S gas around the sensor leading to erroneously high readings.

Supplementary information 2 – Detection of hydrogen di-/polysulfides and glutathione per-/polysulfides

The formation of inorganic and organic persulfides and polysulfides in the reaction of ATTM with reduced (GSH) and oxidized (GSSG) glutathione was monitored by mass spectrometry following alkylation with iodoacetamide (IAM), essentially as described earlier (Bogdándi et al., 2018). 2.5 mmol l^{-1} ATTM or sodium sulfide (Na_2S) were incubated in the absence or presence of 0.5 mmol l^{-1} GSH or GSSG in 10 mmol l^{-1} ammonium phosphate buffer, pH 7.4, at either room temperature or 37°C. Aliquots of these reaction mixtures were removed at 30 mins' and reacted with excess IAM (final concentration 10 mmol l^{-1}) for a further 30 mins at room temperature prior to placement into an autosampler (kept at 4°C) and subsequent mass spectrometric analysis. Due to the unavailability of stable isotope labelled standards for the different polysulfides, no exact concentrations could be calculated; results are therefore reported as 'peak areas'.

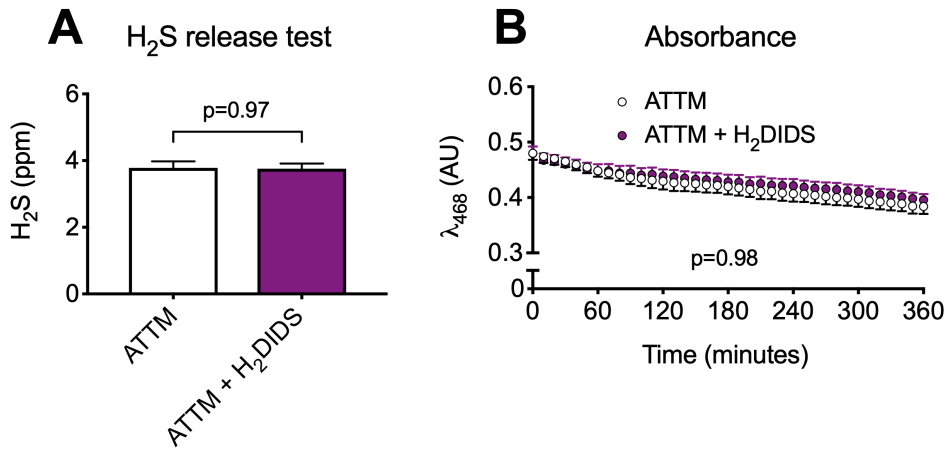
Aliquots of the derivatised reaction solutions were analysed using a Waters Aquity UPLC system coupled to a tandem quadrupole mass spectrometer (Xevo TQ-S, Waters, UK). A mixed-mode column (1.6 µm Modus 100 x 2.2 mm Aqua; Chromatography Direct, UK) kept at 30°C was used for chromatographic separation. Mobile phase A was 5 mmol l^{-1} ammonium formate in water with 0.15% formic acid; mobile phase B was 5 mmol l^{-1} ammonium formate in 95% acetonitrile/5% H_2O with 0.15% formic acid. The following gradient was used: 99% A decreasing to 60% A over 4.5 min, then down to 0% A over 0.5 min and maintained at that level for 1.5 min; the column was then equilibrated back to 99% A over 0.5 min and maintained at 99% A for an additional 1 min. Flow rate was 0.2 ml min^{-1}, and injection volume was 5 µL. Mass spectrometry settings: capillary voltage 3.0 kV, source offset 5 V, desolvation gas flow 800 l h^{-1}, cone gas flow 150 l h^{-1}, nebulizer pressure 7.0 bar, collision gas flow 0.14 ml min^{-1}, desolvation temperature 400°C. The following multiple reaction monitoring (MRM) transitions were used for the detection of IAM-derivatised sulfide and polysulfide species: 149 > 104 (IAM_2-S_1), 181 > 91 (IAM_2-S_2), 213 > 91 (IAM_2-S_3), 245 > 91 (IAM_2-S4), 277 > 91 (IAM_2-S_5), 309 > 91 (IAM_2-S_6) and 341 > 91 (IAM_2-S_7); cone and collision energies were 8 V and 12 V, respectively. MRM transitions for the detection of glutathione and glutathione per-/polysulfides were 365 > 235.5 (GS_1-IAM), 397 > 267.5 (GS_2-IAM), 429 > 299.5 (GS_3-IAM), 461 > 331.5 (GS_4-IAM). Cone and collision energies were 10 V and 12 V, respectively.

Supplementary Fig 1



Supplementary Fig 1. Thiol interactions. (A) H_2S release from ATTM following incubation with reduced (GSH) or oxidised (GSSG) glutathione (n=5). Values recorded are in parts per million (ppm). (B) Degradation of ATTM ± GSH (n=8) or GSSG (n=5) monitored by absorbance at 468 nm (λ_{468}). Untreated samples are n=14. (C) pilot data shows generation of glutathione persulfide and organic polysulfides following incubation of ATTM, the oxomolybdate, $[NH_4]_2MoO_4$ and Na_2S with either GSH or GSSG. Due to lack of authentic standards, data are shown as 'peak areas'. *p<0.05, 1- or 2-way ANOVA followed by Bonferroni's test. Stated p-values are the result of overall ANOVA. AU, arbitrary units;

Supplementary Fig 2



Supplementary Fig 2. Chemical interaction of ATTM with H_2DIDS. (A) H_2S release from ATTM. Standard conditions apply; incubation of ATTM (25 mmol l^{-1}; 100 mmol l^{-1} total sulfur) for 1h at physiological temperature and pH, with or without H_2DIDS (0.5 mmol l^{-1}) (n=6). (B) Degradation of ATTM (175 μmol l^{-1}) ± H_2DIDS (3.5 μmol l^{-1}) (n=6) monitored by absorbance at 468 nm (λ_{468}). Conditions here were designed to replicate an intracellular environment (37°C, pH 6.8). In both experiments, the ratio of ATTM to H_2DIDS reflects that used in the sulfhaemoglobin studies. Statistics: (A) unpaired non-parametric T-test, (B) 2-way ANOVA followed by Bonferroni's test. Stated p-values are the result of the T-test and overall ANOVA. AU, arbitrary units; H_2DIDS, 4'diisothiocyanato-dihydrostilbene- 2,2'-disulfonic acid; ppm, parts per million; λ, wavelength.

Reference

Bogdándi, V., Ida, T., Sutton, T. R., Bianco, C., Ditrói, T., & Koster, G. (2018). Speciation of reactive sulfur species and their reactions with alkylating agents: Do we have any clue about what is present inside the cell? *British Journal of Pharmacology*, 176, 646–670. https://doi.org/ 10.1111/bph.14394