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Ammonium tetrathiomolybdate as a water-soluble and slow-release hydrogen sulfide donor

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ABSTRACT

Ammonium tetrathiomolybdate (TTM) was found to be a slow hydrogen sulfide (H_2S) releasing agent. Its H_2S generation capability in aqueous solutions was confirmed by UV–vis and fluorescence assays. TTM also showed H_2S-like cytoprotective effects in hydrogen peroxide (H_2O_2)-induced oxidative damage in HaCaT cells.

Hydrogen sulfide (H_2S) is newly recognized as a nitric oxide (NO)-like gaseous transmitter that plays regulatory roles in many physiological and pathological processes.[1–5] Endogenous production of H_2S involves both enzymatic pathways (mediated by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST)) and non-enzymatic pathways (from the sulfane sulfur pools). Current knowledge strongly suggests that modulation of H_2S could have potential therapeutic values for certain disease states, including vasodilation, anti-inflammation, anti-oxidation, and down regulation of cellular metabolism under stress.[1–5] Because of this, the search for compounds that can release H_2S and mimic the beneficiary activities of H_2S has become an attractive area in medicinal chemistry.[6–10] So far, a number of H_2S releasing compounds (also known as H_2S donors), such as GYY4137, dithiothiones, *N*-mercapto-based donors, persulfide-based donors, gem-dithiol-based donors, etc., have been reported (Fig. 1).[6–10] These compounds are normally small organic molecules which can be triggered by certain biologically relevant or compatible reactions to release H_2S. Some of them have shown promising bioactivities.[6–10] On the other hand, inorganic molecule-based donors, especially metal complexes, which can slowly hydrolyze to release H_2S have not been reported. Inspired by the fact that sodium nitroprusside (SNP), with the formula $Na_2[Fe(CN)_5NO]$, is a widely used NO donor,[11–13] we suspected that certain sulfide-containing inorganic compounds might be able to serve as interesting H_2S donors in aqueous solutions. Like SNP, the release of H_2S from inorganic molecule-based donors is likely to be the result of simple hydrolysis. As such, the donors would be suitable for many biological studies. Herein we wish to report the discovery of ammonium tetrathiomolybdate (TTM) as an effective inorganic H_2S donor.

TTM, with the formula $(NH_4)_2MoS_4$, is a commonly used building block in the chemistry of molybdenum.[14,15] As an excellent copper chelator, TTM has been used therapeutically in the treatment of copper toxicosis, especially for Wilson's disease.[16] It was previously noted that under strong acidic condition (5% H_2SO_4) H_2S could be generated from TTM.[17] We first wondered if TTM could produce H_2S under mild and biologically friendly conditions,

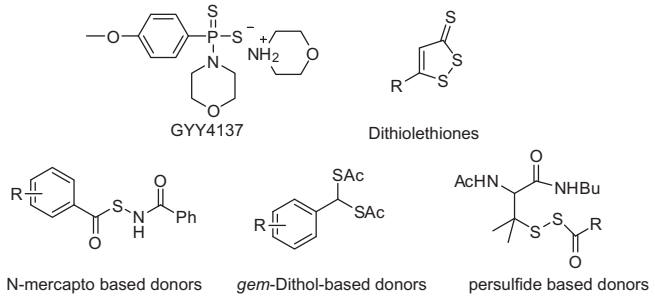

Figure 1. Representative H_2S donors.

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especially under physiological pH. To this end, we measured H_2S release from TTM in four different pH buffers (5, 6, 7.4, and 8). Normally the release of H_2S from the donors can be determined by the standard methylene blue (MB) method.[18] However, a strong acidic condition was involved in this method. As it is known that acidic media would facilitate TTM hydrolysis, we envisioned that the standard MB method was not appropriate. In this study, a zinc-sulfide precipitation based MB method was used.[19] This method should avoid the false positive signals caused by acid-promoted TTM hydrolysis. Briefly, the solutions of 500 μM TTM were freshly prepared in phosphate buffers under different pH. At different time intervals, aliquots were taken to Eppendorf vials containing a mixture of zinc acetate and NaOH solution. After 15 min, solid ZnS was formed and collected by centrifugation. ZnS was then treated by a MB cocktail. The resulted H_2S concentrations were obtained by UV–vis measurements and calculated based on a calibration curve. As shown in Figure 2, we observed immediate H_2S formation in all of these TTM solutions. The level of H_2S in pH 5 was significantly higher than the levels in other pH values. In all solutions we found H_2S concentrations were maintained in a stable level for a long time (up to 15 h). These results indicate TTM is a stable and slow release H_2S donor.

To further confirm the production of H_2S from TTM in buffers, a H_2S gas trapping experiment was designed. As shown in Figure 3, a solution of TTM was placed in a sealed glass vial. An Eppendorf vial containing a solution of WSP-5, a H_2S specific fluorescent probe,[20] was also placed in the vial to trap the evaporated H_2S from the TTM solution. After incubation at 37 °C for 3 h, the trapping solution was diluted and the fluorescence intensity (excitation at 502 nm, emission at 525 nm) was measured. Both the positive and negative controls (using the standard Na_2S solution or pure buffer) were also carried out using the same procedure for comparison. As shown in Figure 4, TTM and Na_2S led to significant fluorescence increases in the trapping solutions while the pure buffer did not cause any detectable fluorescence increase. The pH dependence of H_2S release from TTM was also noted as higher fluorescence was observed in pH 5 than in other pHs. These results further demonstrated H_2S generation from TTM in buffers.

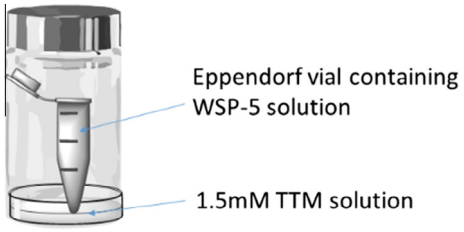

Figure 3. H_2S gas trapping set-up.

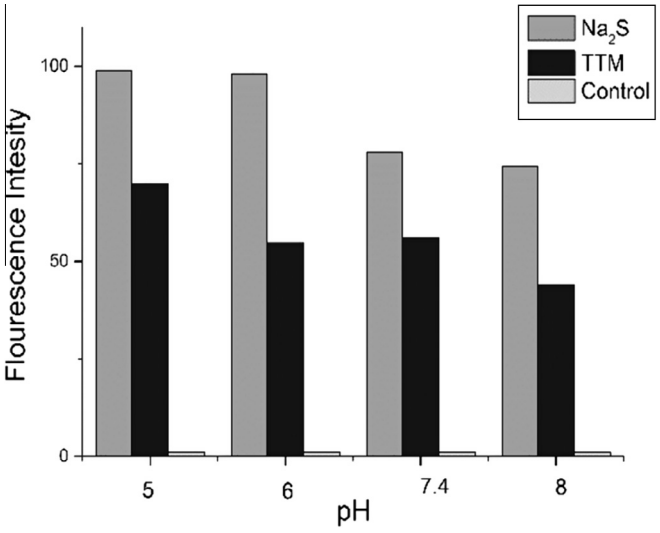

Figure 4. H_2S release of TTM detected by indirect fluorescent assay.

One of the most well-studied biological functions of H_2S is its cytoprotective effects under oxidative stress. Being a H_2S donor, TTM was expected to have similar effects. We then applied TTM

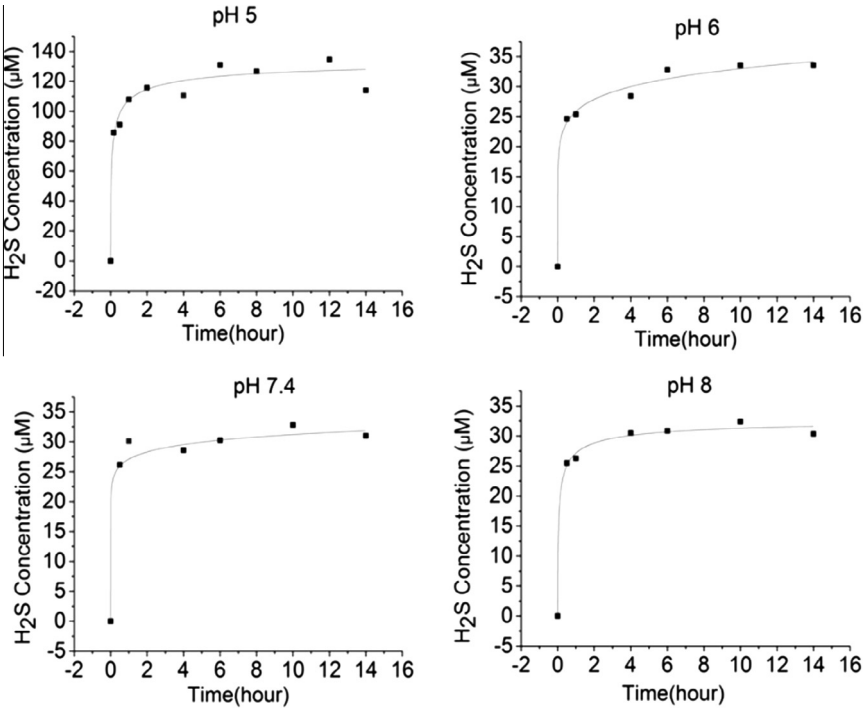

Figure 2. H_2S release from TTM under different pHs.