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Cyclic Sulfenyl Thiocarbamates Release Carbonyl Sulfide (COS) and Hydrogen Sulfide (H₂S) Independently in Thiol-Promoted Pathways

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Abstract

Hydrogen sulfide (H₂S) is an important signaling molecule that provides protective activities in a variety of physiological and pathological processes. Among the different types of H₂S donor compounds, thioamides have attracted attention due to prior conjugation to non-steroidal anti-inflammatory drugs (NSAIDs) to access H₂S-NSAID hybrids with significantly-reduced toxicity, but the mechanism of H₂S release from thioamides remains unclear. Herein, we reported the synthesis and evaluation of a class of thioamide-derived sulfenyl thiocarbamates (**SulfenylTCMs**) that function as a new class of H₂S donors. These compounds are efficiently activated by cellular thiols to release carbonyl sulfide (COS), which is quickly converted to H₂S by carbonic anhydrase (CA). In addition, through mechanistic investigations we establish that COS-independent H₂S release pathways are also operative. In contrast to the parent thioamide-based donors, the **SulfenylTCMs** exhibit excellent H₂S releasing efficiencies of up to 90% and operate through mechanistically well-defined pathways. In addition, we demonstrate that the sulfenyl thiocarbamate group is readily attached to common NSAIDs, such as naproxen, to generate **YZ-597** as an efficient H₂S-NSAID hybrid, which we demonstrate releases H₂S in cellular environments. Taken together, this new class of H₂S donor motifs provide an important platform for new donor development.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information. H₂S release data, NMR experiments, cell imaging, and spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>

Introduction

Gasotransmitters, such as nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S), are small gaseous signaling molecules that are produced endogenously and transmit chemical signals within the organism, tissues, and cells by acting on specific targets.^{1–3} H₂S, which is the youngest member of the gasotransmitter family, is generated from cysteine (Cys) and homocysteine (Hcy) by cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE), and cysteine aminotransferase (CAT)/3-mercaptopyruvate sulfur transferase (3-MST), which work either individually or in concert to regulate H₂S levels under physiological conditions.^{4–6} Once generated, H₂S plays important roles in a variety of physiological and pathological events.^{7–10}

To deliver H₂S in complex environments, numerous H₂S releasing agents (H₂S donors) have been developed. These donors function as important chemical tools to both mimic H₂S biosynthesis and also to investigate H₂S chemistry and biology in contextually-rich environments. Although sodium sulfide (Na₂S) and sodium hydrosulfide (NaSH) are the most commonly-used sources of exogenous H₂S, both of these compounds release H₂S instantly and spontaneously in aqueous media, making the controlled release of sulfide unfeasible.^{11–12} Ideal H₂S donors should only release sulfide upon activation and deliver H₂S with slower, but controllable kinetics. In response to this need, chemists have developed different types of H₂S donors in the last decade, which can be activated by different triggers, such as hydrolysis,^{13–15} cellular thiols,^{16–26} light,^{27–31} pH modulation,^{32–33} and enzymes^{34–35} (Figure 1).^{36–43} Among these donors, aryl thioamides have attracted attention due to their synthetic simplicity and ease of incorporation into common pharmaceutical compounds.²⁴ For example, when compared to regular non-steroidal anti-inflammatory drugs (NSAID), thioamide-coupled NSAIDs, such as **ATB-346**, have retained promising anti-inflammatory activities while significantly reducing side GI damage, suggesting potential applications of these donors as H₂S-related therapeutics.^{38, 44} Although thioamides provide promising donor motifs, the H₂S releasing efficiency of thioamide-based donors remains at relatively low levels (typically 1 – 2%), and the detailed mechanism of H₂S remains unclear (Figure 1 and Scheme 2 top).

To further diversify available H₂S donor platforms and also to develop mechanistically well-defined donors, our group has recently reported new strategies to access H₂S donors through the intermediate release of carbonyl sulfide (COS). In our initial approach, COS was caged in a self-immolative thiocarbamate system. Upon removal of the protecting group, the cascade decomposition of the thiocarbamate released COS, which is quickly hydrolyzed to H₂S by the ubiquitous mammalian enzyme carbonic anhydrase (CA) with an associated rate constant of $2.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (for bovine CA II) (Scheme 1 top).^{45–47} Following our initial report, our group, as well as others,⁴⁸ have developed a series of COS-based H₂S donors that can be triggered through different mechanisms, such as cellular reactive oxygen species (ROS),^{49–51} esterases,^{46, 52–53} nucleophiles,⁵⁴ light,^{55–57} click chemistry,⁵⁸ and Cys.⁵⁹ More recently, we have broadened our approach to include different activation strategies and core motifs to develop colorimetric⁶⁰ and fluorescent⁶¹ COS-based H₂S donors, such as γ -**KetoTCM-1** and **FLD**, which released COS/H₂S with a concomitant change in optical readout (Scheme 1 bottom).

Advancing from this prior work, we envisioned that hybrid COS-releasing constructs based on thioamide cores could be used to leverage the increased H₂S-releasing efficiency from COS donors with the beneficial properties of thioamides, such as synthetic simplicity and ease of incorporation into common pharmaceutical compounds. Here we report the design, synthesis, evaluation, and mechanistic investigation of thioamide-derived cyclic sulfenyl thiocarbamates (**SulfenylTCMs**, also known as 1,2,4-dithiazolin-3-ones).^{62,63} The **SulfenylTCMs** are stable in aqueous solutions but are activated by cellular thiols to cleave the cyclic disulfide and release COS, which is quickly converted to H₂S by CA. The resultant iminodisulfide intermediate then reacts further with cellular thiols to generate a thioamide, which can further release H₂S (Scheme 2 bottom).

Results and Discussion

Donor synthesis

To test our hypothesis that cyclic **sulfenylTCMs** can function as thiol-activated COS/H₂S donors, we first prepared **sulfenylTCMs** by treating corresponding thioamides with chlorocarbonylsulfenyl chloride (Scheme 3). Briefly, chlorocarbonylsulfenyl chloride (2.0 equiv.) was added to anhydrous THF containing the desired thioamide (1.0 equiv.) at 0 °C. The resultant solution was stirred at room temperature until the completion of the reaction as indicated by TLC. The reaction solution was then concentrated and the **sulfenylTCM** was isolated and purified by flash column chromatography. Five donors with aryl (**SulfenylTCM-1 – 4**) or alkyl (**SulfenylTCM-5**) substituents were prepared, and **SulfenylTCM-1** was selected as the model donor for COS/H₂S releasing and mechanistic evaluations.

GSH-Activated COS/H₂S release from SulfenylTCM-1

To evaluate thiol-activated H₂S delivery from the donor motifs, we used the colorimetric methylene blue (MB) assay to monitor H₂S production from **SulfenylTCM-1** (25 μM) in the presence of GSH (0 – 1000 μM) in PBS buffer (pH 7.4, 10 mM) containing cellularly-relevant concentrations of CA (25 μg/mL). GSH was used as the model thiol trigger due to its cellular abundance (typically 5 – 10 mM) and high nucleophilicity. The MB assay was chosen to measure H₂S production since it has been widely used to detect H₂S from a variety of H₂S donors. Treating **SulfenylTCM-1** in PBS in the absence of GSH failed to provide detectable H₂S signal, indicating negligible spontaneous H₂S delivery from **SulfenylTCM-1**. In the presence of GSH, however, **SulfenylTCM-1** exhibited a dose-dependent COS/H₂S release response (Figure 2). These results demonstrate that **SulfenylTCM-1** is activated by GSH in aqueous buffer, and that the resultant COS is quickly converted to H₂S by CA.

Thiol-activated COS/H₂S release from SulfenylTCM-1

Because disulfide bonds are readily cleaved by thiol species, we anticipated that **SulfenylTCM-1** should be triggered by not only GSH, but also by other cellular thiols, such as Cys, Hcy, *N*-acetylcysteine (NAC), and penicillamine (PEN). To test this hypothesis, we treated **SulfenylTCM-1** (25 μM) with each thiol trigger (500 μM) in PBS buffer (pH 7.4, 10 mM) containing CA (25 μg/mL) and monitored H₂S generation using the MB assay. As

expected, a time-dependent COS/H₂S release was observed in the presence of Cys, Hcy, or NAC, indicating a successful activation of **SulfenylTCM-1**. In comparison, H₂S delivery was significantly reduced (~10% COS/H₂S release) in the presence of PEN, presumably due to the increased steric bulk and resultant decrease in nucleophilicity, which prohibited its reaction with **SulfenylTCM-1** (Figure 3). Taken together, these studies demonstrated that **SulfenylTCM-1** can be activated by a variety of cellular thiol species, such as GSH, Cys, Hcy, NAC and PEN. In addition, COS/H₂S delivery from **SulfenylTCM-1** can be controlled by using different triggers due to their different reactivities towards **SulfenylTCM-1**.

Effects of cellular nucleophiles on COS/H₂S release from **SulfenylTCM-1**

To investigate whether COS/H₂S release can be triggered by other species, **SulfenylTCM-1** (25 μM) was treated with biologically relevant species (500 μM), including oxidized glutathione (GSSG), lysine (Lys), serine (Ser), glycine (Gly), thiosulfate (S₂O₃²⁻), sulfite (SO₃²⁻), and sulfate (SO₄²⁻), in PBS buffer (pH 7.4, 10 mM) containing CA (25 μg/mL) and COS/H₂S release was monitored using the MB assay. Compared to GSH-induced donor activation, which led to 70% H₂S production during a 4-hour reaction period, none of the above species triggered **SulfenylTCM-1** (Figure 4). These studies confirmed that **SulfenylTCM-1** is stable towards common biological nucleophiles and reactive sulfur species, but also highly sensitive towards thiol activation to deliver COS/H₂S.

GSH-Activated COS/H₂S release from Other **SulfenylTCMs**

Having evaluated COS/H₂S production from **SulfenylTCM-1**, we next investigated the COS/H₂S releasing efficiency of other **SulfenylTCMs** (**SulfenylTCM-2 – 5**) using GSH as the model trigger. In these experiments, the **SulfenylTCM** donors (25 μM) were incubated with GSH (500 μM) in PBS buffer (pH 7.4, 10 mM) containing CA (25 μg/mL). As expected, all donors were activated by GSH and COS/H₂S release was detected by the MB assay. Importantly, the rate of COS/H₂S release from these donor motifs was changed by structural modifications, which demonstrates that release rates can be tuned by use of differently-substituted thioamide precursors. For example, even though **SulfenylTCM-1 – 3** showed similar COS/H₂S releasing profile, donors with strong electron withdrawing groups (i.e. CF₃ in **SulfenylTCM-4**) or small alkyl group (i.e. CH₃ in **SulfenylTCM-5**) provided significantly enhanced COS/H₂S release (Figure 5). During the 4-hour time course of these experiments, we were surprised to observe over 100% H₂S release from **SulfenylTCM-4** (130%) and **SulfenylTCM-5** (126%). Although the extra H₂S could potentially be attributed to H₂S release from the 4-methoxythiobenzamide and thioacetamide byproducts, the low H₂S releasing efficiency of thioamide-based H₂S donors suggested to us that an alternative COS-independent H₂S releasing pathway was plausible in these **SulfenylTCM** systems.

To determine whether **SulfenylTCM** donors also released H₂S directly through a COS-independent pathway, we treated **SulfenylTCM-1 – 5** (25 μM) with GSH (500 μM) in PBS (pH 7.4, 10 mM) in the absence of CA. For each donor, direct H₂S release was observed using the MB assay, which confirmed the existence of direct H₂S releasing pathway(s) (Figure 6). Although certain aryl thioamides have been reported previously as Cys-activated H₂S donors, we did not observe H₂S release from thioamides (25 μM), such as thiobenzamide, in the presence of GSH (500 μM) using the MB assay under our conditions

(Figure S1).²⁴ Taken together, these studies demonstrated that **SulfenylTCM** donors can release H₂S through a COS- and thioamide-independent pathway (*vide infra*).

Mechanistic investigation on COS/H₂S release from **SulfenylTCM-1**

To further investigate the operative direct H₂S release mechanism from **SulfenylTCMs**, we next conducted NMR experiments to determine which intermediates and products were formed during the course of the reaction in the absence of CA. We first confirmed the stability of **SulfenylTCM-1** in DMSO-*d*₆/D₂O (9:1) over the course of the standard experiments (Figure S3). Next, we prepared a DMSO-*d*₆/D₂O (9:1) solution containing **SulfenylTCM-1** (10 mM) and 5 equiv. of benzyl mercaptan (BnSH, 50 mM) and monitored the reaction by NMR spectroscopy. This experiment showed that the reaction was complete with full consumption of **SulfenylTCM-1** within 30 min. The two major products of the reaction were thiobenzamide and benzyl disulfide (BnSSBn), which were confirmed by comparison to authentic samples (Figures 7 and S4). In related experiments using substoichiometric amounts of BnSH (0.1 – 0.5 equiv.), we also observed complete **SulfenylTCM-1** consumption, which indicated that the thiol triggers could serve as promoters for **SulfenylTCM** activation (Figures S5 and S6). This observation is consistent with a mechanism in which activation of **SulfenylTCM-1** by BnSH generates reactive thiol intermediates, which would further react with the donor motif to release COS/H₂S. In addition, we also treated **SulfenylTCM-1** (5.0 mM) with GSH (10.0 mM) in DMSO/PBS (pH 7.4, 10 mM) (1:1) for 4 h. Thiobenzamide was then isolated as the final product (yield 98%), which is consistent with our NMR study using BnSH as a model trigger (Figure S7).

Based on the MB measurements and NMR experiments, three main observations drive the mechanistic requirements of donor activation: (1) **SulfenylTCM** donors can be activated by thiols to release COS, which functions as an H₂S precursor in the presence of CA; (2) **SulfenylTCM** donors can also directly release H₂S in the absence of CA; and (3) thiobenzamide and disulfide (RSSR) are the two major products when treating **SulfenylTCM-1** with either substoichiometric or excess RSH (0.1 – 5 equiv.), such as BnSH. According to these results, we proposed donor activation and H₂S releasing pathways in Scheme 4. Briefly, thiol triggers (RSH) can react with the **SulfenylTCM** to cleave the disulfide bond, which results in a dethiocarboxylation to release COS, which is quickly converted to H₂S by CA (COS-dependent pathway). This step also generates iminodisulfide intermediate **1**, which can undergo thiol/disulfide exchange to generate RSSR and thiobenzamide (pathway A). A thiol can also react with iminodisulfide **1** at the electrophilic imine carbon to yield iminothioether **2** and a persulfide (RSSH), which can react further with thiols to generate H₂S (pathway B – COS-independent H₂S releasing pathway). Adding further complexity to this reactivity, the generated H₂S or persulfides could also likely intercept **1** to yield the thiobenzamide product and generate an additional persulfide or polysulfide, respectively. In addition, any persulfide intermediates can also likely react with the donor motif directly to propagate this chain of reactions to yield H₂S.

In our investigations, we did not observe iminothioether **2** directly in our NMR experiments (Figures 7, S4, S5, S6), but we suspected that **2** would likely react directly with H₂S to generate RSH, which would further react with **SulfenylTCM-1**. This general reaction

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scheme supports the role of thiols acting as a promotor for **SulfenylITCM** activation (Figures S5 and S6). To test our hypothesis, we synthesized iminothioether **2** and treated it (10 mM) with NaSH (100 mM) in DMSO-*d*₆/D₂O (9:1). NMR experiments showed the full consumption of **2** by H₂S within 10 min, and the formation of thiobenzamide and RSH as the final products (Figure S8), which is consistent with proposed reaction pathway B. This reactivity suggests that H₂S generated in the system may go on to propagate donor activation. Furthermore, this reactivity is consistent with the recently-reported Cys-triggered H₂S donation from iminothioether derivatives.²⁵ Moreover, our studies here provided useful mechanistic insights on internal H₂S scavenging by iminothioether donor motifs, which also helps to explain the low H₂S releasing efficiency observed in the recently-reported iminothioether donor systems.²⁵

Generation of a COS-hybrid naproxen as new COS/H₂S-releasing NSAID

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To demonstrate the generality of sulfenyl thiocarbamate incorporation into biologically-active platforms, we envisioned that this donor motif could be readily incorporated into compounds used for anti-inflammatory activity, such as non-steroidal anti-inflammatory drugs (NSAIDs). One of the major limitation of NSAIDs is the potential gastrointestinal (GI) and cardiovascular toxicity.^{64–65} Recent efforts to improve the therapeutic profile and reduce the GI toxicity of these drugs have included the generation of a series of H₂S-releasing hybrid NSAIDs (H₂S-NSAIDs), many of which include thioamides (Figure 8a–c).^{38, 66–67} For example, one of the most successful H₂S-NSAIDs that progressed into clinical development is **ATB-346**, a naproxen derivative coupled with thiobenzamide as an H₂S releasing moiety. This hybrid H₂S-NSAID exhibited similar anti-inflammatory activities to naproxen but with significantly-reduced GI damage.^{38, 44, 68} Aligned with this high therapeutic potential, **ATB-346** has advanced to two clinical trials in 2014⁶⁹ and 2017.⁷⁰ One limitation of **ATB-346**, much like other thioamide-based donors, is the poorly-understood H₂S releasing mechanism and the relatively low efficiency of H₂S release, which was reported to be only 10 – 20 μM H₂S from 1 mM of the drug motif.³⁸ We viewed that developing related H₂S-releasing NSAIDs with more efficient and mechanistically-understood donor motifs could provide a useful platform for further leveraging these hybrid H₂S donor / drug system.

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To investigate whether cyclic sulfenyl thiocarbamates could be used to enhance H₂S release from such systems, we prepared **YZ-597** from **ATB-346** (Figure 8d). Our expectation, based on our work described above, was that this compound would be activated much more efficiently than the parent H₂S-NSAID conjugate. Consistent with this hypothesis, when we incubated **YZ-597** (25 μM) in the presence of GSH (500 μM) in PBS buffer (pH 7.4, 10 mM) containing CA (25 μg/mL) and 1 mM CTAB to help solubilize the components we observed rapid and efficient H₂S release (90%). Although H₂S release from **ATB-346** has been reported to be favored in the presence of reducing agents, such as Cys and GSH or in the presence of biological materials, we did not observed efficient H₂S release from **ATB-346** under our experimental conditions (Figure 9).⁶⁸ These data suggest that **YZ-597** may have more accessible H₂S releasing pathways than the thioamide-derived **ATB-346** motif.

Having confirmed H₂S release from **YZ-597** in the presence of GSH, we next investigated whether this conjugate could release H₂S efficiently in live cells. We first incubated HeLa cells with FBS-free DMEM containing the H₂S-responsive fluorescent probe **SF7-AM** (5 μM), and after washing then added either **YZ-597** (50 μM) or vehicle.⁷¹ As shown in Figure 10, the HeLa cells displayed negligible H₂S fluorescent signal in the absence of **YZ-597**, suggesting minimal endogenous H₂S levels. In comparison, cells treated with **YZ-597** revealed a strong fluorescent signal, indicating that **YZ-597** was activated to release H₂S in a cellular environment. We also treated HeLa cells with the donor motif **SulfenylTCM-1** under the identical condition and observed similar fluorescence enhancement (Figure S9). Taken together, we view that **YZ-597** and related sulfenyl thiocarbonate motifs can provide a useful platform for investigating the action of H₂S-hybrid NSAID and related pharmacologically-active compounds.

Conclusions

Thioamide-derived sulfenyl thiocarbamates function as thiol-activated COS/H₂S donors. These compounds can be activated by cellular thiol species, such as GSH, Cys, Hcy, and NAC, to deliver H₂S. We demonstrated that the H₂S-releasing efficiency of **SulfenylTCMs** is significantly enhanced in comparison to the parent thioamide compounds. Moreover, COS and H₂S release from **SulfenylTCM** compounds is mechanistically well-defined, which provides the ability to tune release parameters. Leveraging this design platform, we also prepared the COS/H₂S-NSAID, **YZ-597**, which is built off of the **ATB-346** platform, and demonstrated the efficient H₂S release both *in vitro* and in live cells from **YZ-597**. In addition, we anticipate that **YZ-597** and related compounds will also serve as a promising chemical tools to provide insights into the relationship between H₂S release and efficacy of H₂S-hybrid compounds. Further investigations into the activity of **YZ-597** and related H₂S hybrids are currently ongoing in our laboratory.

Experimental Section

Materials and Methods

Reagents were purchased from Sigma Aldrich, Tokyo Chemical Industry (TCI), Fisher Scientific, and VWR and used directly as received. Carbonic anhydrase (CA) from bovine erythrocytes was purchased from Sigma-Aldrich (C2624). Silica gel (SiliaFlash F60, Silicycle, 230–400 mesh) was used for column chromatography. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA). ¹H, ¹⁹F, and ¹³C{¹H} NMR spectra were recorded on Bruker 500 MHz NMR instrument at the indicated frequencies. Chemical shifts are reported in ppm relative to residual protic solvent resonances. Mass spectrometric measurements were performed by the University of Illinois, Urbana Champaign MS facility, or on a Xevo Waters ESI LC/MS instrument. Methylene blue absorbance was monitored by an UV–vis spectrometer (Cary 100, Agilent Technologies, Santa Clara, California, USA) in PBS buffer. **SF7-AM**,⁷¹ **ATB-346**,⁷² and iminothioether **2**²⁵ were synthesized by following the literature report. HeLa cells were purchased from ATCC (Manassas, Virginia, USA). Cell imaging experiments were

performed on a Leica DMi8 fluorescence microscope, equipped with an Andor Zyla 4.2+ sCMOS detector.

Synthesis

SulfenylTCM-1. Thiobenzamide (135 mg, 1.00 mmol) was dissolved in anhydrous THF (15 mL) followed by the addition of chlorocarbonylsulfenyl chloride (262 mg, 2.00 mmol) at 0 °C. The resultant solution was stirred at 0 °C for 10 min, after which the ice bath was removed, and the reaction mixture was stirred at r.t. for 1 h. The reaction solution was then concentrated under vacuum and the crude product was purified by column chromatography.

SulfenylTCM-1 was isolated as yellow solid (70%). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.16 (d, *J* = 10.0 Hz, 2H), 7.80 (d, *J* = 10.0 Hz, 1H), 7.66 (t, *J* = 10.0 Hz, 2H). ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm): 189.3, 186.1, 135.5, 131.4, 130.3, 128.5. IR (cm⁻¹): 1680, 1655, 1592, 1501, 1479, 1445, 1306, 1235, 1086, 1065, 923, 766, 683, 669. HRMS *m/z* [M+H]⁺ calcd for [C₈H₆NOS₂]⁺ 195.9891; found 195.9891.

SulfenylTCM-2 was prepared from 4-methoxythiobenzamide by following the procedure described above (47 mg, 41% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.14 (d, *J* = 10.0 Hz, 2H), 7.17 (d, *J* = 10.0 Hz, 2H), 3.91 (s, 3H). ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm): 188.2, 185.8, 165.3, 130.9, 123.9, 115.6, 56.4. IR (cm⁻¹): 2963, 2835, 1668, 1597, 1574, 1521, 1484, 1421, 1308, 1245, 1168, 1078, 1023, 925, 828, 666. HRMS *m/z* [M+H]⁺ calcd for [C₉H₈NO₂S₂]⁺ 225.9996; found 225.9999.

SulfenylTCM-3 was prepared from 4-chlorothiobenzamide by following the procedure described above (91 mg, 81% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.17 (d, *J* = 10.0 Hz, 2H), 7.73 (d, *J* = 10.0 Hz, 2H). ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm): 188.1, 186.0, 140.3, 130.3, 130.2. IR (cm⁻¹): 3068, 3033, 1660, 1590, 1504, 1477, 1400, 1306, 1281, 1239, 1178, 1008, 827, 666. HRMS *m/z* [M+H]⁺ calcd for [C₈H₅ClNOS₂]⁺ 229.9505; found 229.9497.

SulfenylTCM-4 was prepared from 4-trifluoromethylthiobenzamide by following the procedure described above (104 mg, 79% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.35 (d, *J* = 10.0 Hz, 2H), 8.01 (d, *J* = 10.0 Hz, 2H). ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm): 188.0, 186.0, 134.9, 129.4, 127.1, 125.0, 122.9. ¹⁹F NMR (470 MHz, DMSO-*d*₆) δ (ppm): -61.8. IR (cm⁻¹): 3047, 1661, 1517, 1493, 1408, 1330, 1305, 1190, 1171, 1118, 1064, 1011, 924, 845, 669. HRMS *m/z* [M+H]⁺ calcd for [C₉H₅F₃NOS₂]⁺ 263.9765; found 263.9760.

SulfenylTCM-5 was prepared from thioacetamide by following the procedure described above (35 mg, 40% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 2.79 (s, 3H). ¹³C{¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm): 192.9, 187.0, 22.9. IR (cm⁻¹): 1662, 1516, 1494, 1165, 1071, 1000, 668, 628, 589. HRMS *m/z* [M+H]⁺ calcd for [C₃H₄NOS₂]⁺ 133.9734; found 133.9735.

YZ-597 was prepared from **ATB-346** by following the procedure described above (105 mg, 87% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.19 (d, *J* = 5.0 Hz, 2H), 7.88 (s, 2H), 7.86 (s, 1H), 7.54 (d, *J* = 5.0 Hz, 1H), 7.34 (s, 2H), 7.32 (s, 1H), 7.19 (d, *J* = 10.0 Hz, 1H),

4.29 (q, $J = 5.0$ Hz, 1H), 3.88 (s, 3H), 1.63 (d, $J = 10.0$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, DMSO- d_6) δ (ppm): 188.2, 186.0, 172.8, 157.8, 155.9, 135.3, 134.0, 130.3, 129.7, 129.0, 127.8, 126.7, 126.4, 123.5, 119.4, 106.3, 55.7, 45.0, 18.8. IR (cm^{-1}): 1742, 1677, 1662, 1485, 1412, 1206, 1165, 1135, 1117, 1087, 1028, 893, 853, 844, 814. HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $[\text{C}_{22}\text{H}_{18}\text{NO}_4\text{S}_2]^+$ 424.0677; found 424.0657.

H₂S Release from SulfenylTCMs in PBS

A **SulfenylTCM** stock solution (50.0 μL , 10.0 mM in DMSO) was added to 20.0 mL of PBS (pH 7.40, 10.0 mM) containing CA (25.0 $\mu\text{g/mL}$) in a 25-mL scintillation vial. A thiol stock solution (0.100 M in H₂O) was then added to generate the desired thiol working concentrations as shown in Figures 2, 3, and 5. For H₂S release in the absence of CA, the measurement was set up by following the procedure as described above in PBS with no CA addition (Figure 6). For H₂S release from **YZ-597**, the measurement was set up by following the procedure as described above in PBS containing CA (25.0 $\mu\text{g/mL}$) and CTAB (1.00 mM) (Figure 9). Next, 0.300 mL aliquots of the reaction mixture were transferred to UV cuvettes containing 0.300 mL of MB cocktail (0.060 mL zinc acetate (1.00% w/v), 0.120 mL FeCl₃ (30.0 mM in 1.20 M HCl), and 0.120 mL *N,N*-dimethyl-*p*-phenylene diamine (20.0 mM in 7.20 M HCl)) at different time points. The absorbance at 670 nm was then measured after 30 min and was converted to H₂S concentration by using the H₂S calibration curve.

Selectivity investigations on H₂S release

A **SulfenylTCM-1** stock solution (50.0 μL , 10.0 mM in DMSO) was added to 20.0 mL of PBS (pH 7.40, 10.0 mM) containing CA (25.0 $\mu\text{g/mL}$) in a 25-mL scintillation vial. An analyte stock solution (100 μL , 100 mM in H₂O) was then added. The reaction was stirred at r.t. for 4 h. Next, 0.300 mL aliquot of the reaction mixture was transferred to UV cuvettes containing 0.300 mL of MB cocktail (0.060 mL zinc acetate (1.00% w/v), 0.120 mL FeCl₃ (30.0 mM in 1.20 M HCl), and 0.120 mL *N,N*-dimethyl-*p*-phenylene diamine (20.0 mM in 7.20 M HCl)). The absorbance at 670 nm was then measured after 30 min and was converted to H₂S concentration by using the H₂S calibration curve.

Mechanism investigations of H₂S release

SulfenylTCM-1 (0.0200 M) and BnSH (0.100 M) solutions were prepared by adding **SulfenylTCM-1** (3.90 mg) and BnSH (12.4 mg) in DMSO- d_6 /D₂O (9:1, 1.00 mL), respectively. Then **SulfenylTCM-1** (0.0200 M, 0.500 mL) was added to BnSH (0.100 M, 0.500 mL) to reach the concentrations of 0.0100 M of **SulfenylTCM-1** and 0.0500 M of BnSH, respectively. The reaction process was then monitored by using a Bruker 500 MHz NMR instrument.

Cellular imaging of H₂S release from YZ-597

HeLa cells were plated in poly-D-lysine coated plates (MatTek) containing 2 mL of DMEM and incubated at 37 °C under 5% CO₂ for 24 h. The confluent cells were washed with PBS and then incubated with **SF7-AM** (5.00 μM) and Hoechst dye (10.0 $\mu\text{g/mL}$) for 5 min. The cells were then washed with PBS and incubated with in FBS-free DMEM in the absence or presence of **YZ-597** (50.0 μM) for 30 min. Prior to imaging, cells were washed with PBS

and bathed in 2 mL of PBS. Cell imaging was performed on a Leica DMI8 fluorescent microscope using DIC for bright field and a standard DAPI and GFP filter cubes for fluorescence imaging, respectively. The scale bar represents 50 μ m.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Wang R, Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol. Rev* 2012, 92 (2), 791–896. [PubMed: 22535897]
2. Szabo C, Hydrogen sulphide and its therapeutic potential. *Nat. Rev. Drug. Discov* 2007, 6 (11), 917–935. [PubMed: 17948022]
3. Filipovic MR; Zivanovic J; Alvarez B; Banerjee R, Chemical Biology of H₂S Signaling through Persulfidation. *Chem. Rev* 2018, 118 (3), 1253–1337. [PubMed: 29112440]
4. Wallace JL; Wang R, Hydrogen sulfide-based therapeutics: exploiting a unique but ubiquitous gasotransmitter. *Nat. Rev. Drug Discov* 2015, 14 (5), 329–345. [PubMed: 25849904]
5. Kabil O; Banerjee R, Enzymology of H₂S biogenesis, decay and signaling. *Antioxid. Redox. Signal* 2014, 20 (5), 770–782. [PubMed: 23600844]
6. Bruce King S, Potential biological chemistry of hydrogen sulfide (H₂S) with the nitrogen oxides. *Free Radic. Biol. Med* 2013, 55, 1–7. [PubMed: 23165065]
7. Szabo C, Gasotransmitters in cancer: from pathophysiology to experimental therapy. *Nat. Rev. Drug. Discov* 2016, 15 (3), 185–203. [PubMed: 26678620]
8. Cao X; Bian JS, The Role of Hydrogen Sulfide in Renal System. *Front Pharmacol.* 2016, 7, 385–396. [PubMed: 27803669]
9. Cao X; Cao L; Ding L; Bian JS, A New Hope for a Devastating Disease: Hydrogen Sulfide in Parkinson's Disease. *Mol. Neurobiol* 2018, 55 (5), 3789–3799. [PubMed: 28536975]
10. Kimura H, Signaling molecules: hydrogen sulfide and polysulfide. *Antioxid. Redox. Signal* 2015, 22 (5), 362–376. [PubMed: 24800864]
11. DeLeon ER; Stoy GF; Olson KR, Passive loss of hydrogen sulfide in biological experiments. *Anal. Biochem* 2012, 421 (1), 203–207. [PubMed: 22056407]
12. Whiteman M; Li L; Rose P; Tan CH; Parkinson DB; Moore PK, The effect of hydrogen sulfide donors on lipopolysaccharide-induced formation of inflammatory mediators in macrophages. *Antioxid. Redox. Signal* 2010, 12 (10), 1147–1154. [PubMed: 19769459]
13. Li L; Whiteman M; Guan YY; Neo KL; Cheng Y; Lee SW; Zhao Y; Baskar R; Tan CH; Moore PK, Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. *Circulation* 2008, 117 (18), 2351–2360. [PubMed: 18443240]
14. Park CM; Zhao Y; Zhu Z; Pacheco A; Peng B; Devarie-Baez NO; Bagdon P; Zhang H; Xian M, Synthesis and evaluation of phosphorodithioate-based hydrogen sulfide donors. *Mol. Biosyst* 2013, 9 (10), 2430–2434. [PubMed: 23917226]
15. Zhu Y; Romero EL; Ren X; Sanca AJ; Du C; Liu C; Karim ZA; Alshbool FZ; Khasawneh FT; Zhou J; Zhong D; Geng B, Clopidogrel as a donor probe and thioenol derivatives as flexible promoiety for enabling H₂S biomedicine. *Nat. Commun* 2018, 9 (1), 3952. [PubMed: 30262863]
16. Zhao Y; Wang H; Xian M, Cysteine-activated hydrogen sulfide (H₂S) donors. *J. Am. Chem. Soc* 2011, 133 (1), 15–17. [PubMed: 21142018]

17. Zhao Y; Bhushan S; Yang C; Otsuka H; Stein JD; Pacheco A; Peng B; Devarie-Baez NO; Aguilar HC; Lefer DJ; Xian M, Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury. *ACS Chem. Biol* 2013, 8 (6), 1283–1290. [PubMed: 23547844]
18. Zhao Y; Yang C; Organ C; Li Z; Bhushan S; Otsuka H; Pacheco A; Kang J; Aguilar HC; Lefer DJ; Xian M, Design, Synthesis, and Cardioprotective Effects of N-Mercapto-Based Hydrogen Sulfide Donors. *J. Med. Chem* 2015, 58 (18), 7501–7511. [PubMed: 26317692]
19. Zhao Y; Kang J; Park CM; Bagdon PE; Peng B; Xian M, Thiol-activated gem-dithiols: a new class of controllable hydrogen sulfide donors. *Org. Lett* 2014, 16 (17), 4536–4539. [PubMed: 25141097]
20. Foster JC; Powell CR; Radzinski SC; Matson JB, S-arylothiooximes: a facile route to hydrogen sulfide releasing compounds with structure-dependent release kinetics. *Org. Lett* 2014, 16 (6), 1558–1561. [PubMed: 24575729]
21. Cerda MM; Hammers MD; Earp MS; Zakharov LN; Pluth MD, Applications of Synthetic Organic Tetrasulfides as H₂S Donors. *Org. Lett* 2017, 19 (9), 2314–2317. [PubMed: 28440074]
22. Cerda MM; Zhao Y; Pluth MD, Thionoesters: A Native Chemical Ligation-Inspired Approach to Cysteine-Triggered H₂S Donors. *J. Am. Chem. Soc* 2018, 140 (39), 12574–12579. [PubMed: 30230325]
23. Cerda MM; Newton TD; Zhao Y; Collins BK; Hendon CH; Pluth MD, Dithioesters: simple, tunable, cysteine-selective H₂S donors. *Chem. Sci* 2019, 10 (6), 1773–1779. [PubMed: 30842844]
24. Martelli A; Testai L; Citi V; Marino A; Pugliesi I; Barresi E; Nesi G; Rapposelli S; Taliani S; Da Settimo F; Breschi MC; Calderone V, Arylthioamides as H₂S Donors: l-Cysteine-Activated Releasing Properties and Vascular Effects in Vitro and in Vivo. *ACS Med. Chem. Lett* 2013, 4 (10), 904–908. [PubMed: 24900583]
25. Barresi E; Nesi G; Citi V; Piragine E; Piano I; Taliani S; Da Settimo F; Rapposelli S; Testai L; Breschi MC; Gargini C; Calderone V; Martelli A, Iminothioethers as Hydrogen Sulfide Donors: From the Gasotransmitter Release to the Vascular Effects. *J. Med. Chem* 2017, 60 (17), 7512–7523. [PubMed: 28799763]
26. Roger T; Raynaud F; Bouillaud F; Ransy C; Simonet S; Crespo C; Bourguignon MP; Villeneuve N; Vilaine JP; Artaud I; Galardon E, New biologically active hydrogen sulfide donors. *ChemBioChem* 2013, 14 (17), 2268–2271. [PubMed: 24115650]
27. Devarie-Baez NO; Bagdon PE; Peng B; Zhao Y; Park CM; Xian M, Light-induced hydrogen sulfide release from “caged” gem-dithiols. *Org. Lett* 2013, 15 (11), 2786–2789. [PubMed: 23697786]
28. Fukushima N; Ieda N; Sasakura K; Nagano T; Hanaoka K; Suzuki T; Miyata N; Nakagawa H, Synthesis of a photocontrollable hydrogen sulfide donor using ketoprofenate photocages. *Chem. Commun* 2014, 50 (5), 587–589.
29. Venkatesh Y; Das J; Chaudhuri A; Karmakar A; Maiti TK; Singh NDP, Light triggered uncaging of hydrogen sulfide (H₂S) with real-time monitoring. *Chem. Commun* 2018, 54 (25), 3106–3109.
30. Xiao Z; Bonnard T; Shakouri-Motlagh A; Wylie RAL; Collins J; White J; Heath DE; Hagemeyer CE; Connal LA, Triggered and Tunable Hydrogen Sulfide Release from Photogenerated Thiobenzaldehydes. *Chem. Eur. J* 2017, 23 (47), 11294–11300. [PubMed: 28489258]
31. Woods JJ; Cao J; Lippert AR; Wilson JJ, Characterization and Biological Activity of a Hydrogen Sulfide-Releasing Red Light-Activated Ruthenium(II) Complex. *J. Am. Chem. Soc* 2018, 140 (39), 12383–12387. [PubMed: 30230336]
32. Kang J; Li Z; Organ CL; Park CM; Yang CT; Pacheco A; Wang D; Lefer DJ; Xian M, pH-Controlled Hydrogen Sulfide Release for Myocardial Ischemia-Reperfusion Injury. *J. Am. Chem. Soc* 2016, 138 (20), 6336–6339. [PubMed: 27172143]
33. Wu J; Li Y; He C; Kang J; Ye J; Xiao Z; Zhu J; Chen A; Feng S; Li X; Xiao J; Xian M; Wang Q, Novel H₂S Releasing Nanofibrous Coating for In Vivo Dermal Wound Regeneration. *ACS Appl. Mater. Interfaces* 2016, 8, 27474–27481. [PubMed: 27504858]
34. Shukla P; Khodade VS; SharathChandra M; Chauhan P; Mishra S; Siddaramappa S; Pradeep BE; Singh A; Chakrapani H, “On demand” redox buffering by H₂S contributes to antibiotic resistance revealed by a bacteria-specific H₂S donor. *Chem. Sci* 2017, 8 (7), 4967–4972. [PubMed: 28959420]

35. Zheng Y; Yu B; Ji K; Pan Z; Chittavong V; Wang B, Esterase-Sensitive Prodrugs with Tunable Release Rates and Direct Generation of Hydrogen Sulfide. *Angew. Chem. Int. Ed* 2016, 55 (14), 4514–4518.
36. Kashfi K; Olson KR, Biology and therapeutic potential of hydrogen sulfide and hydrogen sulfide-releasing chimeras. *Biochem. Pharmacol* 2013, 85 (5), 689–703. [PubMed: 23103569]
37. Zhao Y; Biggs TD; Xian M, Hydrogen sulfide (H₂S) releasing agents: chemistry and biological applications. *Chem. Commun* 2014, 50 (80), 11788–11805.
38. Szabo C; Papapetropoulos A, International Union of Basic and Clinical Pharmacology. CII: Pharmacological Modulation of H₂S Levels: H₂S Donors and H₂S Biosynthesis Inhibitors. *Pharmacol. Rev* 2017, 69 (4), 497–564. [PubMed: 28978633]
39. Bora P; Chauhan P; Pardeshi KA; Chakrapani H, Small molecule generators of biologically reactive sulfur species. *RSC Adv.* 2018, 8 (48), 27359–27374.
40. Hartle MD; Pluth MD, A practical guide to working with H₂S at the interface of chemistry and biology. *Chem. Soc. Rev* 2016, 45 (22), 6108–6117. [PubMed: 27167579]
41. Xu S; Hamsath A; Neill DL; Wang Y; Yang CT; Xian M, Strategies for the Design of Donors and Precursors of Reactive Sulfur Species. *Chem. Eur. J* 2019, 25 (16), 4005–4016. [PubMed: 30372550]
42. Powell CR; Dillon KM; Matson JB, A review of hydrogen sulfide (H₂S) donors: Chemistry and potential therapeutic applications. *Biochem. Pharmacol* 2018, 149, 110–123. [PubMed: 29175421]
43. Zhao Y; Pacheco A; Xian M, Medicinal Chemistry: Insights into the Development of Novel H₂S Donors. *Handb. Exp. Pharmacol* 2015, 230, 365–388. [PubMed: 26162844]
44. Wallace JL; Caliendo G; Santagada V; Cirino G, Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). *Br. J. Pharmacol* 2010, 159 (6), 1236–1246. [PubMed: 20128814]
45. Steiger AK; Pardue S; Kevil CG; Pluth MD, Self-Immolative Thiocarbamates Provide Access to Triggered H₂S Donors and Analyte Replacement Fluorescent Probes. *J. Am. Chem. Soc* 2016, 138 (23), 7256–7259. [PubMed: 27218691]
46. Levinn CM; Steiger AK; Pluth MD, Esterase-Triggered Self-Immolative Thiocarbamates Provide Insights into COS Cytotoxicity. *ACS Chem. Biol* 2019, 14 (2), 170–175. [PubMed: 30640440]
47. Haritos VS; Dojchinov G, Carbonic anhydrase metabolism is a key factor in the toxicity of CO₂ and COS but not CS₂ toward the flour beetle *Tribolium castaneum* Coleoptera : Tenebrionidae. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 2005, 140 (1), 139–147.
48. Steiger AK; Zhao Y; Pluth MD, Emerging Roles of Carbonyl Sulfide in Chemical Biology: Sulfide Transporter or Gasotransmitter? *Antioxid. Redox. Signal* 2018, 28 (16), 1516–1532. [PubMed: 28443679]
49. Zhao Y; Henthorn HA; Pluth MD, Kinetic Insights into Hydrogen Sulfide Delivery from Caged-Carbonyl Sulfide Isomeric Donor Platforms. *J. Am. Chem. Soc* 2017, 139 (45), 16365–16376. [PubMed: 29056039]
50. Zhao Y; Pluth MD, Hydrogen Sulfide Donors Activated by Reactive Oxygen Species. *Angew. Chem. Int. Ed* 2016, 55 (47), 14638–14642.
51. Chauhan P; Jos S; Chakrapani H, Reactive Oxygen Species-Triggered Tunable Hydrogen Sulfide Release. *Org. Lett* 2018, 20 (13), 3766–3770. [PubMed: 29927612]
52. Chauhan P; Bora P; Ravikumar G; Jos S; Chakrapani H, Esterase Activated Carbonyl Sulfide/ Hydrogen Sulfide (H₂S) Donors. *Org. Lett* 2017, 19 (1), 62–65. [PubMed: 27996277]
53. Steiger AK; Marcatti M; Szabo C; Szczesny B; Pluth MD, Inhibition of Mitochondrial Bioenergetics by Esterase-Triggered COS/H₂S Donors. *ACS Chem. Biol* 2017, 12 (8), 2117–2123. [PubMed: 28613823]
54. Powell CR; Foster JC; Okyere B; Theus MH; Matson JB, Therapeutic Delivery of H₂S via COS: Small Molecule and Polymeric Donors with Benign Byproducts. *J. Am. Chem. Soc* 2016, 138 (41), 13477–13480. [PubMed: 27715026]
55. Sharma AK; Nair M; Chauhan P; Gupta K; Saini DK; Chakrapani H, Visible-Light-Triggered Uncaging of Carbonyl Sulfide for Hydrogen Sulfide (H₂S) Release. *Org. Lett* 2017, 19 (18), 4822–4825. [PubMed: 28872885]

56. Stacko P; Muchova L; Vitek L; Klan P, Visible to NIR Light Photoactivation of Hydrogen Sulfide for Biological Targeting. *Org. Lett* 2018, 20, 4907–4911. [PubMed: 30062897]
57. Zhao Y; Bolton SG; Pluth MD, Light-Activated COS/H₂S Donation from Photocaged Thiocarbamates. *Org. Lett* 2017, 19 (9), 2278–2281. [PubMed: 28414240]
58. Steiger AK; Yang Y; Royzen M; Pluth MD, Bio-orthogonal “click-and-release” donation of caged carbonyl sulfide (COS) and hydrogen sulfide (H₂S). *Chem. Commun* 2017, 53 (8), 1378–1380.
59. Zhao Y; Steiger AK; Pluth MD, Cysteine-activated hydrogen sulfide (H₂S) delivery through caged carbonyl sulfide (COS) donor motifs. *Chem. Commun* 2018, 54 (39), 4951–4954.
60. Zhao Y; Steiger AK; Pluth MD, Colorimetric Carbonyl Sulfide (COS)/Hydrogen Sulfide (H₂S) Donation from gamma-Ketothiocarbamate Donor Motifs. *Angew. Chem. Int. Ed* 2018, 57 (40), 13101–13105.
61. Zhao Y; Cerda MM; Pluth MD, Fluorogenic hydrogen sulfide (H₂S) donors based on sulfenyl thiocarbonates enable H₂S tracking and quantification. *Chem. Sci* 2019, 10 (6), 1873–1878. [PubMed: 30842856]
62. Chen L; Thompson TR; Hammer RP; Barany G, Synthetic, Mechanistic, and Structural Studies Related to 1,2,4-Dithiazolidine-3,5-dione. *J. Org. Chem* 1996, 61 (19), 6639–6645. [PubMed: 11667534]
63. Barany G; Musier-Forsyth K; Xu Q; Chen L; Hammer RP Sulfurization of phosphorus-containing compounds. WO Patent 1997041130, 6 11, 1997.
64. Grosser T; Fries S; FitzGerald GA, Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J. Clin. Invest* 2006, 116 (1), 4–15. [PubMed: 16395396]
65. Wallace JL, Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol. Rev* 2008, 88 (4), 1547–1565. [PubMed: 18923189]
66. Caliendo G; Cirino G; Santagada V; Wallace JL, Synthesis and biological effects of hydrogen sulfide (H₂S): development of H₂S-releasing drugs as pharmaceuticals. *J. Med. Chem* 2010, 53 (17), 6275–6286. [PubMed: 20462257]
67. Chan MV; Wallace JL, Hydrogen sulfide-based therapeutics and gastrointestinal diseases: translating physiology to treatments. *Am. J. Physiol. Gastrointest. Liver Physiol* 2013, 305 (7), G467–G473. [PubMed: 23868410]
68. Gemici B; Elsheikh W; Feitosa KB; Costa SK; Muscara MN; Wallace JL, H₂S-releasing drugs: anti-inflammatory, cytoprotective and chemopreventative potential. *Nitric Oxide* 2015, 46, 25–31. [PubMed: 25461269]
69. ClinicalTrials.gov.; , Study To Assess Safety, Tolerability And PK Of ATB-346 In Healthy Subjects.
70. ClinicalTrials.gov.; , To Compare the Gastrointestinal Safety of a 14-Day Oral Dosing Regimen of ATB-346 to Sodium Naproxen in Healthy Subjects.
71. Lin VS; Lippert AR; Chang CJ, Cell-trappable fluorescent probes for endogenous hydrogen sulfide signaling and imaging H₂O₂-dependent H₂S production. *Proc. Natl. Acad. Sci. U S A* 2013, 110 (18), 7131–7135. [PubMed: 23589874]
72. Li M; Li J; Zhang T; Zhao Q; Cheng J; Liu B; Wang Z; Zhao L; Wang C, Syntheses, toxicities and anti-inflammation of H₂S-donors based on non-steroidal anti-inflammatory drugs. *Eur. J. Med. Chem* 2017, 138, 51–65. [PubMed: 28646655]

Figure 1.
Selected synthetic H₂S donors.

Figure 2.

COS/H₂S Release from **SulfenylTCM-1** (25 μM) in the presence of 0 μM (black), 250 μM (red), 500 μM (blue), and 1000 μM (green) GSH. The experiments were performed in triplicate and results are expressed as mean $\pm$ S.D. ($n = 3$).

Figure 3. Thiol-dependent (500 μM) $\text{COS}/\text{H}_2\text{S}$ release from **SulfenylTCM-1** (25 μM). The experiments were performed in triplicate and results are expressed as mean $\pm$ S.D. ($n = 3$).

Figure 4. COS/ H_2S Release from **SulphenylTCM-1** (25 μM) in the presence of cellular nucleophiles (500 μM). H_2S concentration was measured after 4-h incubation. The experiments were performed in triplicate and the results were expressed as mean $\pm$ S.D. ($n = 3$).

Figure 5. COS/ H_2S release from **SulfenylTCM-1 – 5** (25 μM) in the presence of GSH (500 μM) in PBS (pH 7.4, 10 mM) containing CA (25 $\mu\text{g/mL}$). The experiments were performed in triplicate and the results are expressed as mean $\pm$ S.D. ($n = 3$).

Figure 6.

COS-Independent H_2S release from **SulfenylTCM-1 – 5** (25 μM) in the presence of GSH (500 μM) in PBS (pH 7.4, 10 mM). The experiments were performed in triplicate and the results were expressed as mean $\pm$ S.D. ($n = 3$).

Figure 7. $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of the reaction between **SulfenylTCM-1** (red star) and BnSH (green square). Thiobenzamide (purple circle) and BnSSBn (blue triangle) were identified as major products using authentic samples.

Figure 8. Representative thioamide-containing H_2S -NSAIDs, such as (a) **GIC-1001**, (b) **NBS-1121**, and (c) **ATB-346**, and (d) COS/ H_2S -NSAID **YZ-597**.

Figure 9. GSH (500 μM)-triggered H_2S release from **YZ-597** or **ATB-346** (25 μM). The experiments were performed in triplicate and results are expressed as mean $\pm$ S.D. ($n = 3$).

Figure 10.

H₂S Delivery from **YZ-597** in HeLa cells. HeLa cells were treated with SF7-AM (5 μ M) and Hoechst (10 μ g/mL) for 5 min. After removal of extracellular **SF7-AM** and Hoechst, cells were incubated in FBS-free DMEM in the absence (Top row) or presence (Bottom row) of **YZ-597** (50 μ M) for 30 min. Cells were then washed and imaged in PBS. Scale bar: 50 μ m.

Common activation scheme for COS-releasing donors **Selected examples of COS/H₂S Donors** **Scheme 1.**Examples of COS-based H₂S donors.

Scheme 2.

(Top) Cys-Activated H_2S release from thioamides. (Bottom) Proposed thiol-triggered COS/ H_2S release from SulfenylTCMs.

Scheme 3.
Synthesis of sulfenyl thiocarbamates.

Scheme 4.

Proposed mechanism of thiol-triggered COS/H₂S release from SulfenylTCM-1.