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Development and Application of COS-based Donors for H₂S Delivery

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Conspectus

In addition to nitric oxide and carbon monoxide, hydrogen sulfide (H₂S) has been recently recognized as an important biological signaling molecule with implications in a wide variety of processes including vasodilation, cytoprotection, and neuromodulation. In parallel to the growing number of reports highlighting the biological impact of H₂S, interest in developing H₂S donors as both research tools and potential therapeutics has led to the growth of different H₂S-releasing strategies. Many H₂S investigations in model systems use direct inhalation of H₂S gas or aqueous solutions of NaSH or Na₂S, however such systems do not mimic endogenous H₂S production. This stark contrast drives the need to develop better sources of caged H₂S to be used in biological systems. To address these limitations, different small organosulfur donor compounds have been prepared that release H₂S in the presence of specific activators or triggers. Such compounds, however, often lack suitable H₂S-depleted control compounds, which limits the use of these compounds in probing the effects of H₂S directly. To address these needs, our group has pioneered the development of carbonyl sulfide (COS) releasing compounds as a new class of H₂S donor motifs. Inspired by a commonly-used carbamate prodrug scaffold, our approach utilizes self-immolative thiocarbonates to access controlled release of COS, which is rapidly converted to H₂S by the ubiquitous enzyme carbonic anhydrase (CA). In addition, this design enables access to key control compounds that release CO₂/H₂O rather than COS/H₂S, which enables delineation of the effects of COS/H₂S from the organic donor byproducts.

In this Account, we highlight a library of first-generation COS/H₂S donors based on self-immolative thiocarbonates developed in our lab and also highlight challenges related to H₂S donor development. We showcase the release of COS in the presence of specific triggers and activators including biological thiols and bioorthogonal reactants for targeted applications. We also demonstrate the design and development of a series of H₂O₂ / reactive oxygen species (ROS)-triggered donors and show that such compounds can be activated by endogenous levels of ROS production. Utilizing approaches in bio-orthogonal activation, we establish that donors functionalized with an *o*-nitrobenzyl photocage can enable access to light-activated donors. Similar to endogenous production by cysteine catabolism, we also prepared a cysteine-selective COS donor activated by a Strongin-ligation mechanism. In efforts to help delineate potential differences in the chemical biology of COS and H₂S, we also report a simple esterase-activated

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donor, which demonstrated fast COS-releasing kinetics and inhibition of mitochondrial respiration in BEAS-2B cells. Additional investigations revealed that COS release rates and cytotoxicity correlated directly within this series of compounds with different ester motifs. In more recent and applied applications of this H₂S donation strategy, we also highlight the development of donors that generate either a colorimetric or fluorescence optical response upon COS release. Overall, the work described in this Account outlines the development and initial application of a new class of H₂S donors, which we anticipate will help to advance our understanding of the rapidly emerging chemical biology of H₂S and COS.

Graphical Abstract

Introduction

Initially disregarded as a toxic and foul-smelling gas,¹ hydrogen sulfide (H₂S) has recently emerged as an important biological signaling molecule commonly known as a “gasotransmitter”² with major implications in biological systems.^{3,4} H₂S is produced endogenously by native enzymes including cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) primarily by cysteine catabolism.⁵ As a gasotransmitter, H₂S can react directly with biological targets or activate specific pathways in the cardiovascular, neuronal, and gastrointestinal systems.⁶ These signaling pathways have also been observed in diseases states including diabetes,⁷ atherosclerosis,⁸ and Parkinson’s disease.⁹ Notably, H₂S-mediated signaling has been implicated in vital life processes including modulation of neurotransmission,¹⁰ vasodilation,¹¹ and cytoprotection against reactive oxygen species (ROS) (Figure 1).¹² As examples of the role of H₂S in different model systems, treatment of murine hippocampal slices with H₂S resulted in the enhancement of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses and induction of long-term potentiation, an important neuronal process during memory formation.¹³ Similar to nitric oxide, a known vasodilator, administration of H₂S to the cardiovascular system directly activates ATP-sensitive potassium (K_{ATP}) channels triggering membrane hyperpolarization promoting an overall decrease in arterial blood pressure.¹⁴ The addition of H₂S to mouse embryonic fibroblasts from a CSE knockout model displaying elevated levels of oxidative stress was shown to halt cellular senescence by persulfidation of Keap1 and activation of Nrf2 leading to increased production of reduced glutathione (GSH), a potent antioxidant.¹⁵ In addition to these observations, a continually-growing number of reports of H₂S-mediated signaling collectively highlight the biological significance of H₂S. This growth has inspired the development of small molecules capable of releasing H₂S termed “donors” under physiologically-relevant conditions at rates comparable to endogenous production with goals of harnessing the potential benefits of H₂S as both a research and therapeutic tool.¹⁶

The controlled delivery of H₂S has been a long-standing challenge due to the inherent chemical properties of H₂S. At physiological pH, the weak acidity of H₂S ($pK_a \sim 7.0$) results in a speciation of ~70% hydrosulfide anion (SH⁻) and ~30% H₂S gas. In the presence of oxygen, and especially in the presence of redox-active metals, H₂S is readily oxidized and leads to the formation of polysulfides,¹⁷ thus complicating the quantification of active H₂S concentrations. To avoid the direct use of H₂S gas, most biological studies have used sodium hydrosulfide (NaSH) and sodium sulfide (Na₂S) as convenient sources of H₂S. The addition of these inorganic sulfide salts to a buffered aqueous solution, however, results in a rapid, almost instantaneous increase in H₂S concentration followed by a gradual decrease due to volatilization of H₂S gas.¹⁸ This fast release of H₂S is in stark contrast to the rate of H₂S production by CBS and CSE measured under similar conditions.¹⁹ These factors drive the need to develop alternative sources of H₂S which better mimic the rate of endogenous H₂S production. To address this problem, a number of synthetic, small molecule H₂S donors including diallyl trisulfide (DATS)²⁰ and GYY4137²¹ have been reported. Despite the wide use of these donors, a lack of tunability and appropriate control compounds limits their use in further probing the physiological effects of H₂S. To address these concerns, donors that release H₂S at varying rates in response to specific stimuli including hydrolysis, thiols, and light have been prepared.^{22–25}

As an alternative approach to previously reported donors that release H₂S directly, we were inspired by the conversion of carbonyl sulfide (COS) by carbonic anhydrase (CA) to H₂S.²⁶ COS is the most abundant sulfur-containing gas in Earth's atmosphere, and we as well as others have recently leveraged COS as vehicle for H₂S delivery.²⁷ Currently, enzymatic pathways for the mammalian biosynthesis of COS have not been identified, but a number of different metalloenzymes that can convert COS to H₂S, most notably, the ubiquitous mammalian enzyme CA. A primary physiological role of CA is regulation of blood pH by conversion of CO₂ to bicarbonate ($K_{cat}/K_M = 8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for bovine CA-II), but as a relatively promiscuous enzyme, CA can also metabolize COS to H₂S and CO₂, with high catalytic efficiency ($K_{cat}/K_M = 2.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ for bovine CA-II).^{28–31}

In 2016, we reported a new approach to access H₂S donors by leveraging the efficient hydrolysis of COS to H₂S by CA.³² We drew inspiration from the widely-employed strategy of using triggerable self-immolative carbamates to deliver a payload in response different stimuli (Figure 2a). Because such scaffolds extrude CO₂ as a byproduct of the self-immolative decomposition, we reasoned that replacing the carbamate core with a thiocarbamate would result in COS release. In this design, the caged-thiocarbamates can be engineered to respond to specific biologically-relevant stimuli to deliver COS, which in turn is rapidly converted to H₂S by CA (Figure 2b,c).

The high modularity of this scaffold allows for a “plug and play” approach to H₂S donor design, in which both the trigger and the payload can be readily modified to accomplish different goals (Figure 3a). Importantly, the analogous carbamates, which release CO₂ rather than COS, serve as key H₂S-depleted control compounds that can help to separate the effects of the organic byproducts from that of COS/H₂S release. Additionally, the triggerless control, which maintains the thiocarbamate core but lacks the self-immolative triggering group, provides an additional control compound that helps to account for any effects

observed as a result of the thiocarbamate moiety. In our first application of this general design, we reported self-immolative thiocarbamates in the development of the first analyte-replacement COS/H₂S fluorescent probe (Figure 3b). With an azide as the triggering group and methyl rhodol as the payload, treatment with NaSH yielded both COS/H₂S release and a fluorescent turn-on response.³² Importantly, this design provided a first step toward addressing the challenge of analyte consumption in activity-based systems. We have since expanded the strategy of triggered COS/H₂S release to encompass a wide range of triggering events and stimuli (Figure 3c).

Stimuli Responsive COS-based H₂S Donors

In an early application of stimulus-responsive COS/H₂S donors, we developed systems in which a boronate ester, which is converted to a phenol by ROS, was used as the trigger (Figure 4a).³³ This system combined the known role of boronate esters as ROS scavengers with the cytoprotective effects of H₂S to access enhanced cytoprotection against ROS. Using an H₂S-selective electrode, we demonstrated the H₂O₂-dependent release of COS/H₂S from the thiocarbamate, but not from carbamate or triggerless control compounds, in the presence of CA. We also established the ability of these H₂O₂-activated donors to release COS/H₂S in live cell environments. For example, treatment of HeLa cells incubated with an H₂S-selective fluorescent probe and the ROS-activated donor exhibited an H₂S-derived fluorescence response when treated with exogenous H₂O₂. Similarly, endogenous levels ROS produced by stimulation with phorbol myristate acetate (PMA) could also trigger COS/H₂S release from the donors, as demonstrated in RAW 264.7 cells. Moreover, the thiocarbamate donors showed significant cytoprotective effects against exogenous H₂O₂ in HeLa cells. The carbamate control compound also proved slightly cytoprotective, although to a much lesser extent than the sulfide-releasing donors (Figure 4b), which was likely due to partial H₂O₂ consumption by the boronate trigger. These results highlight the benefit of having key control compounds to fully disentangle the observed biological effects of the release H₂S from that of other donor components or byproducts. A further systematic study of analogous boronate-containing molecules with variable COS-releasing motifs, including *O*- and *S*-alkyl thiocarbamates, *O*- and *S*-alkyl thiocarbonates, and dithiocarbonates, demonstrated the broad applicability and tunability of this platform in triggerable COS/H₂S delivery.³⁴

The self-immolative thiocarbamate COS donor scaffold has also been used in common bio-orthogonal activation mechanisms, such as photoactivation. In proof-of-concept studies, we reported the first light-activated COS/H₂S donor equipped with a photocleavable *o*-nitrobenzyl group. Upon irradiation ($\lambda = 365$ nm), the benzyl alcohol is cleaved *via* a Norrish type II mechanism, revealing one equivalent of COS and an aniline payload (Figure 5). The rate of H₂S release from this system was found to increase with the addition of electron-donating methoxy substituents on the *o*-nitrobenzyl group consistent with previous findings.³⁵ Building from this work, light-triggered COS release has also been recently reported using BODIPY-derived photolabile groups.^{36,37}

In vivo, H₂S is produced primarily through cysteine catabolism. In an effort to better mimic the conditions of endogenous H₂S production, we applied the established chemistry of the

Strongin ligation to prepare a cysteine-selective COS/H₂S donor. The mechanism of COS release proceeds through nucleophilic attack by cysteine into an acrylate, followed by subsequent cyclization by the pendant amine, and finally in elimination to uncage the thiocarbamate moiety (Figure 6). Due to the requirement of a nearby amine in the mechanism, this donor has an inherent selectivity towards cysteine over other biological thiols including reduced glutathione (GSH). The sulfide release of this donor was shown in bEnd.3 cells using an H₂S-selective fluorescent probe.³⁸

One limitation of many triggerable donor scaffolds is the consumption of biological nucleophiles to initiate release of the desired product, thus perturbing the cellular homeostasis. We envisioned using an enzymatically-triggered reaction as a potential solution, thus enabling activation without depleting the levels of cellular nucleophiles.^{39,40} By appending a small ester to the 4-hydroxybenzyl alcohol core, exposure to intracellular esterases should reveal the corresponding phenolate, which undergoes the same 1,6-self-immolative cascade to generate COS (Figure 7a). Employing a thiocarbamate with a *t*-butyl ester trigger and a *p*-tolyl payload yielded fast COS/H₂S donors as determined by a H₂S-selective electrode in the presence of porcine liver esterase (PLE). Further studies revealed that this donor led to almost complete cell death even at the low concentration of 10 μ M in BEAS2B, whereas neither the triggerless or H₂S-depleted control compounds showed any toxicity at those concentrations. Additionally, neither GYY4137 or AP39, two commonly used H₂S donors were toxic at these levels. Most surprisingly, the thiocarbamate was much more cytotoxic than Na₂S, which is often described to be toxic due to the immediate bolus of H₂S released under physiological conditions (Figure 7b). These results highlight the crucial need for adequate control compounds to delineate observed activities of COS/H₂S from those attributable to organic byproducts of donor activation.³⁹

The unique toxicity profile of the *t*-butyl ester thiocarbamate led us to hypothesize that either the specific subcellular localization of the compound caused cell death, or that a build-up of COS itself directly inhibited mitochondrial bioenergetics. To investigate the latter hypothesis, we prepared a series of esterase-triggered COS donors equipped with esters of varying sizes (Figure 7c).⁴¹ The rate of small ester cleavage by esterases is likely faster than the rate of COS hydrolysis by CA (up to $5.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ compared to $2.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ for bovine CA II), which could lead to a potentially toxic buildup of COS in the cell.²⁹ Changing the size of an ester significantly changes the rate of cleavage by esterases, and we proposed that donors with small esters would exhibit high levels of cytotoxicity, whereas those with bulkier groups would have little effect on cell viability.⁴² Consistent with this hypothesis, we demonstrated that the rate of ester hydrolysis directly correlates with the observed cytotoxicity in HeLa cells, supporting the idea that COS may function as more than a simple H₂S shuttle (Figure 7d). Despite the toxicity of the small ester donors, this report outlined a suite of COS donors with tunable rates of release, the utility of which was highlighted with fluorescent cell-imaging of the cyclohexyl ester thiocarbamate.⁴¹ A similar series of *S*-alkyl thiocarbamate esterase-triggered COS donors were reported by the Chakrapani group, which display comparable toxicity at 50 μ M in human breast cancer MCF-7 cells, however rates of H₂S release were found to be slightly slower.⁴⁰

One challenge associated with the 1,6-self-immolative thiocarbamate scaffold is the release of an electrophilic para-quinone methide. As one approach to address this limitation, and also to demonstrate the compatibility of our approach with common biorthogonal chemistry,⁴³ we developed a “click-and-release” donor, in which COS release is triggered through an inverse-electron demand Diels Alder reaction (IEDDA) with a *trans*-cyclooctene moiety fused to a thiocarbamate (Figure 8). Experiments with both bovine and sheep plasma and blood proved that endogenous levels of CA are sufficient for the hydrolysis of the released COS to H₂S.⁴⁴

COS-based H₂S Donors with Optical Readouts

To assess the validity of our donors *in vitro*, we utilize spectrophotometric methods of H₂S detection such as the methylene blue assay.⁴⁵ In cells, the use of H₂S-selective fluorescent probes^{46–48} consumes the generated H₂S and interferes with our ability to fully observe the effect of H₂S production on cells. To prevent this undesired analyte consumption, we envisioned coupling a spectroscopic response to COS/H₂S release by appending a COS-releasing moiety to a chromophore which would generate a spectroscopic signal concomitantly with the release of COS. This class of donors provides novel chemical tools to visualize COS/H₂S release by UV/Vis or fluorescence spectroscopy and allows us to minimize the number of external components needed to visualize H₂S release in complex biological systems.

In our initial approach, we designed an analogous self-immolation scaffold which undergoes β -elimination at physiological pH to generate methyl vinyl ketone, COS, and release an amine-based payload (Figure 9a).⁴⁹ The use of *p*-nitroaniline (PNA) as the payload allows for optical monitoring of H₂S release from **γ -KetoTCM1** by measuring the UV/Vis absorbance of PNA ($\lambda_{\text{max}} = 381 \text{ nm}$) (Figure 9b). Importantly, UV-Vis spectroscopy showed that PNA formation correlated directly with H₂S generation measured using the methylene blue assay, which confirmed that the optical response can be used as a direct proxy for H₂S release (Figure 9c).

The incorporation of a spectroscopic handle also allowed us to conduct a series of kinetic experiments to probe the effect of pH on COS/H₂S release from this donor by UV/Vis spectroscopy. At physiological pH, this donor releases H₂S slowly over 30 h with a measured pseudo-first order rate constant (k_{obs}) of $4.52(2) \times 10^{-5} \text{ s}^{-1}$. The rate of H₂S release from this donor can be tuned as a function of pH, and the observed rates of H₂S release in basic solutions (pH 8.0, $k_{\text{obs}} = 12.6(2) \times 10^{-5} \text{ s}^{-1}$) consistent with a mechanism of β -elimination. Additionally, we were able to decrease the rate of H₂S release by installing a methyl group at the β -position resulting in modulation of the β -proton acidity. The addition of bovine serum albumin (5 mg/mL) led to a significant increase in the rate of H₂S release at pH 7.4 ($k_{\text{obs}} = 81(3) \times 10^{-5} \text{ s}^{-1}$) suggesting that this donor could function in complex biological environments and benefit from protein microenvironments. In further support of biological compatibility, the addition of biological nucleophiles including cysteine, GSH, and lysine did not impact H₂S release. Moreover, H₂S release from **γ -KetoTCM1** was observed in HeLa cells, as evidenced by the fluorescence response from an H₂S-responsive probe.

To improve optical signal to be more compatible with biological samples, we also developed fluorescent turn-on donors that become fluorescent after release of COS/H₂S. To accomplish this goal, we relied on the reactivity of sulfenyl thiocarbonates towards thiols to generate a disulfide, COS, and alcohol-based payload (Figure 10a). By a simple, one-step procedure, we prepared a small library of fluorescein-caged sulfenyl thiocarbonates to serve as fluorescent, COS-based H₂S donors with **FLD-1** serving as the model fluorescent donor (Figure 10b).⁵⁰ The addition of excess cysteine (100 μM) to 10 μM **FLD-1** in the presence of carbonic anhydrase at pH 7.4 resulted in a fluorescent enhancement of over 500-fold consistent with the formation of fluorescein upon consumption of the donor motif (Figure 10c). Using a monofunctionalized sulfenyl thiocarbonate derivative to simplify the reaction kinetics, we demonstrate the formation of fluorescein monitored by fluorescence spectroscopy can be directly correlated to H₂S release measured by the methylene blue assay and confirms the validity of this approach to prepare fluorescent H₂S/COS donors. The release of H₂S from **FLD-1** was found to occur exclusively in the presence of thiols including cysteine and GSH over other biological nucleophiles including lysine, serine, and H₂O₂. To assess the biological compatibility of **FLD-1**, we examined the activation of this donor by endogenous thiols and concomitant H₂S release by use of 7-azido-4-methylcoumarin (**C7-Az**), an H₂S-selective fluorescent probe in HeLa cells. The treatment of HeLa cells with **FLD-1** (50 μM) resulted in a fluorescent turn-on of **C7-Az** and fluorescent signal corresponding to the formation of fluorescein (Figure 10c). An overlay of both channels reveals an even cellular distribution suggesting good uptake of **FLD-1** and confirms the compatibility of this donor in live cells. Taken together, **γ-KetoTCM-1** and **FLD-1** are the first examples of COS-based H₂S donors with an incorporated optical readout and provide visual chemical tools for probing the biological effects of H₂S.

Perspective and Outlook

The donors covered in this account can collectively be viewed as a toolbox that chemists, biologists, and physiologists can use to probe the chemical biology of H₂S under various conditions and begin to assess the biological impacts of COS. To both further our studies and to advance the field, we envision further investigating the potential roles of COS in sulfur biology. Many of the reported COS-based H₂S donors reported by our group and others have been based primarily on the self-immolative thiocarbamate scaffold. Despite the high modularity, the generation of the quinone methide byproduct is often overlooked. The absence of cytotoxicity in our experiments suggests that short-term exposure to this leads to minimal negative effects, but prior reports suggest that chronic exposure may lead to electrophilic stress,⁵¹ which adds an additional variable to biological investigations. This potential issue drives the need to develop future generations of COS-based H₂S donors that lack electrophilic byproducts. Additionally, further investigation are needed into the potential direct toxicity of COS. We have shown that smaller esterase-triggered COS donors exhibit significant cytotoxicity at concentrations as low as 10 μM, whereas the COS-depleted controls, Na₂S, and other small-molecule direct H₂S donors have no effect. This observation raises the question of whether COS has activity independent of that of H₂S. Although we have demonstrated that the cytotoxicity of small molecule esterase-triggered donors correlates directly with the rate of COS release, fully disentangling the effects of

COS delivery from the physiological effects of H₂S is a complex problem, and remains an unmet challenge in the field. Furthermore, although there are many different isoforms of CA, little is known about the different reactivity toward COS and CO₂, with available data showing that the commonly-used bovine CA-II has a significantly higher catalytic efficiency toward its native substrate CO₂ ($8 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$) than for COS ($2.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$).^{28,29} There is a relative dearth of knowledge about the activities of different CA isoforms toward COS, and how the subcellular localizations of the enzyme isoforms effects the observed toxicity of different COS donors.

Another outstanding challenge is that of COS detection. Although COS can be detected through GC-MS analysis or other spectroscopic methods, there are currently no simple methods available for the detection of COS directly in aqueous solution, which significantly limits the ability to accurately study COS in biological systems. For example, although COS has been detected in the headspace of porcine coronary artery and cardiac muscle, with the current technology it cannot be conclusively determined whether that COS was from mammalian or bacterial origin.⁵² To truly advance the field of biological COS research, solution-phase COS probes and detection methods need to be developed. With the rapidly growing interest in COS as both a vehicle for H₂S delivery and as a distinct biomolecule, we anticipate that these knowledge gaps will be filled through the collective efforts of the gasotransmitter research community, and that a new category of COS-based targeted tools and therapeutics will emerge.

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Biographies

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Matthew M. Cerda received his B.S. in Chemistry from SUNY College at Potsdam in 2015. He is currently a Ph.D. candidate at University of Oregon in lab of Prof. Michael D. Pluth developing chemical tools to study reactive sulfur species including H₂S, COS, and organic polysulfides.

Michael D. Pluth is an Associate Professor in the Department of Chemistry and Biochemistry at the University of Oregon. Mike earned his B.S. degree in Chemistry and Mathematics in 2004 from the University of Oregon. He received his Ph.D. in 2008 from UC Berkeley working under the mentorship of Profs. Robert Bergman and Kenneth Raymond. After postdoctoral research at MIT with Prof. Stephen Lippard as an NIH Pathways to Independence fellow, Mike joined the UO faculty in 2011. His lab focuses on different

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Figure 1.
Representative physiological processes involving H_2S and associated mechanisms of action.

Figure 2.

(a) Design and mechanism of self-immolative carbamates and (b) self-immolative thiocarbamates. (c) Conversion of COS to H₂S by CA in the presence of acetazolamide at varying concentrations measured by a sulfide-selective electrode.

(a) Mechanism of self-immolation and COS/H₂S release **(b) Analyte-replacement fluorescent probes** **(c) Representative self-immolative COS donors and triggers** **Figure 3.**

(a) Mechanism of self-immolation and subsequent conversion of COS to H₂S by CA. (b) Development of analyte-replacement fluorescent probes. (c) Current examples self-immolation-based COS/H₂S donors reported to date by our lab.

Figure 4.

(a) Representative reaction scheme and mechanism for H₂O₂-triggered COS/H₂S release. (b) Cytotoxicity of H₂O₂ in RAW 264.7 cells, Cytotoxicity of Bpin-triggered thiocarbamate donor, Bpin-triggered carbamate control, and triggerless thiocarbamate control at various concentrations in RAW 264.7 cells when co-incubated with 100 μM H₂O₂. (c) Imaging ROS-scavenging upon stimulation with PMA in RAW 264.7 cells.

Figure 5.
Mechanism of self-immolation from photocleavable thiocarbamate COS/H₂S donors.

Figure 6. Mechanism of COS-release from **OA-CysTCM-1** in the presence of cysteine with subsequent hydrolysis of COS to H₂S by carbonic anhydrase.

Figure 7. Altered cytotoxicity via steric modulation for esterase-triggered COS/H₂S donors. (a) Mechanism of self-immolation of esterase-triggered COS donors. (b) Library of different ester size thiocarbamates prepared. (c) Inhibition of mitochondrial bioenergetics with the tert-Butyl triggered COS donor in BEAS2B cells. (d) The relationship between COS release rate and observed cytotoxicity at 100 μ M in HeLa cells for a library of different esterase-triggered COS donors of varying ester size.

Figure 8. Mechanism of 'click-and-release' biorthogonal COS donor, and measured H_2S release in the presence of mammalian blood and plasma, without exogenous CA.

Figure 9.

(a) Mechanism of $\text{COS}/\text{H}_2\text{S}$ release from γ -KetoTCM1 (b) Conditions for measuring H_2S release from γ -KetoTCM1 (c) Measurement of PNA formation over time by UV/Vis spectroscopy (d) Correlation between measured $[\text{H}_2\text{S}]$ and PNA formation by the methylene blue assay and UV/Vis spectroscopy.

Figure 10.

(a) Mechanism of thiol-mediated, COS/H₂S release from sulfenyl thiocarbonates. (b) Structure of **FLD-1** and release of H₂S from **FLD-1** (10 μM) in the presence of cysteine (100 μM, 10 equiv.) in the presence of carbonic anhydrase in 10 mM PBS (pH 7.4) monitored by fluorescence spectroscopy ($\lambda_{\text{ex}} = 490 \text{ nm}$, $\lambda_{\text{em}} = 500\text{--}650 \text{ nm}$). (c) Imaging of cellular H₂S release from **FLD-1** (50 μM) in HeLa cells.