Organic LETTERS

Letter

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S-Aroylthiooximes: A Facile Route to Hydrogen Sulfide Releasing Compounds with Structure-Dependent Release Kinetics

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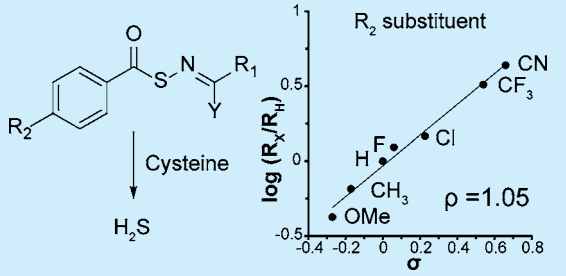
Supporting Information

ABSTRACT: We report the facile preparation of a family of *S*-aroylthiooxime (SATO) H_2S donors, which are synthesized via a click reaction analogous to oxime formation between *S*-aroylthiohydroxylamines (SATHAs) and aldehydes or ketones. Analysis of cysteine-triggered H_2S release revealed structure-dependent release kinetics with half-lives from 8−82 min by substitution of the SATHA ring. The pseudo-first-order rate constants of substituted SATOs fit standard linear free energy relationships (ρ = 1.05), demonstrating a significant sensitivity to electronic effects.



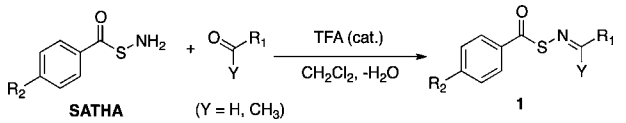
Gasotransmitters such as hydrogen sulfide (H_2S) are endogenous signaling gases that are enzymatically produced and have specific physiological functions,[1] and delivery of these gasotransmitters can be exploited for therapeutic applications.[2] The therapeutic potential of H_2S is vast, with promising preclinical studies conducted on several diseases and disorders. For example, H_2S exhibits cardioprotection through vasorelaxation,[3] suppresses oxidative stress,[4] reduces inflammation in the brain,[5] and protects the liver in ischemia-reperfusion events,[6] among other effects. Unfortunately, H_2S lags behind the other gasotransmitters (NO and CO) in studies on its physiological role and translational potential. Versatile H_2S-releasing functional groups are needed to unlock this untapped potential and elevate the status of this gasotransmitter.

The study of H_2S physiology is accomplished through the use of various H_2S organic and electrochemical sensors[7] in conjunction with H_2S-releasing compounds (we refer to H_2S, HS^-, and S^{2-} collectively here as H_2S, although all three species are in a protonation equilibrium in aqueous solution). Most studies on H_2S physiology and biology have been done with sulfide salts (Na_2S and NaHS). Organic H_2S donors have recently been developed, but most suffer a number of limitations including structural constraints and uncontrolled H_2S release kinetics.[8] The *in vivo* function of H_2S is coupled to its local concentration;[9] therefore, precise control over the rate of H_2S release from donor compounds is paramount.

S-Aroylthiohydroxylamines (SATHAs) have been used in the synthesis of H_2S-releasing *N*-(benzoylthio)benzamides.[8d] As sulfur-containing analogs to acylhydrazides and acylhydroxylamines, thiohydroxylamines are reported to undergo condensation reactions with both aldehydes and ketones to form thiooximes, a functional group that has received little attention in the literature.[10] We hypothesized that *S*-aroylthiooximes (SATOs) might react with cysteine to generate H_2S in a similar manner to previously reported compounds.[8d] Given the simplicity and robust nature of oxime and hydrazone-forming reactions, SATO formation could provide a method for attaching an H_2S-releasing functionality to many types of compounds under mild conditions. Herein, we report a synthetic strategy for accessing a variety of H_2S-releasing compounds via a thiooxime click reaction analogous to oxime formation. Additionally, we explore the reactivity and H_2S-releasing capacity of this unique functional group.

In order to evaluate our hypothesis, we synthesized a series of SATOs from substituted SATHAs and commercially available aldehydes and ketones (Scheme 1). SATHAs were prepared by previously described methods with a range of substituents.[11] Thiooxime formation reactions were conducted at equimolar concentrations of SATHA and aldehyde/ketone in CH_2Cl_2 in the presence of a catalytic quantity of trifluoroacetic acid and molecular sieves.

Scheme 1. Synthesis of SATOs

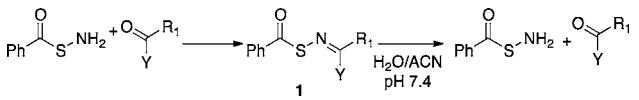


The versatility of thiooxime formation was demonstrated by mixing unsubstituted SATHA (R_2 = H) with several aldehydes and ketones (Table 1) to generate compounds **1a**−**1j**. Aldehyde-derived SATOs (**1a**−**1g**) were synthesized with high conversions and high isolated yields. Ketone-derived SATOs (**1h**−**1j**) were somewhat more difficult to make. Condensation of SATHA with a number of aliphatic aldehydes and ketones was attempted; however, only one aliphatic SATO, derived from pivalaldehyde (**1g**), could be isolated due to rapid

Received: December 16, 2013
Published: February 27, 2014

dx.doi.org/10.1021/ol500385a | *Org. Lett.* 2014, 16, 1558−1561

Table 1. Conversion and Hydrolysis Kinetics of Substituted SATOs



compd	R_1, Y	% conversion[a]	$t_{1/2}$ hydrol.[b] (h)
1a	Ph, H	>99	44
1b	*p*-Ph-F, H	>99	76
1c	*p*-Ph-COOH, H	>99	58
1d	*p*-Ph-OCH_3, H	90	57
1e	furanyl, H	>99	221
1f	cinnanmyl, H	>99	118
1g	$C(CH_3)_3$, H	74	1.0
1h	Ph, CH_3	66	199
1i	*p*-Ph-F, CH_3	87	266
1j	*p*-Ph-OCH_3, CH_3	37	194

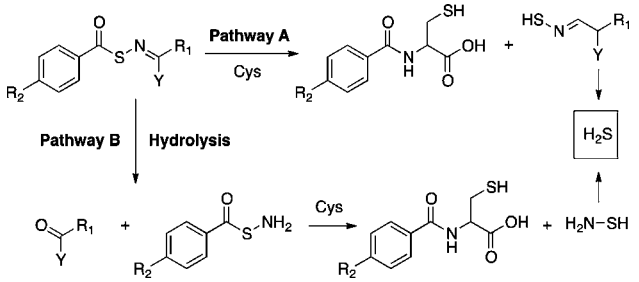
[a]Conversion to SATO as determined by relative integration of thiooxime to aldehyde/ketone signals by ^{1}H NMR. [b]Hydrolysis conducted in 20% (v/v) ACN in PBS (pH = 7.4) at rt.

hydrolysis. We also prepared SATOs with substituents (R_2) on the SATHA ring at near complete conversion.

For the set of ketones studied (**1h**–**1j**), conversion increases with increasing electron withdrawal. These results are consistent with well-known linear free energy relationships.[12] The maximum conversion attained for several substituted SATOs is summarized in Table 1.

Two pathways can be envisioned for generating H_2S from SATOs (Scheme 2). Pathway A involves addition of the

Scheme 2. Possible Routes of H_2S Generation



cysteine thiol to the SATO acyl group followed by rapid S→N acyl transfer in a step similar to native chemical ligation.[13] In this case, the arylidenethiooxime would form, which could decompose to generate the original ketone or aldehyde along with H_2S and NH_3, as has been previously noted for similar substrates.[8d] Pathway B describes an initial hydrolysis step, which would generate the SATHA and the ketone or aldehyde used to make the SATO. (In preliminary experiments, unsubstituted SATHA released H_2S in the presence of cysteine, leading to the same products as proposed for Pathway A.)

While both pathways lead to H_2S release, Pathway A would be more desirable for functionalizing substrates to make prodrugs, where H_2S would be released simultaneously with the drug. Considering the tunable hydrolysis kinetics of the analogous oxime functional group, Pathway B could be discouraged by controlling steric and electronic factors of the SATO bond. Additionally, the rate of H_2S release could be precisely controlled by manipulating the electronics of the SATHA ring because a likely rate-limiting step would be the breakdown or formation of a tetrahedral intermediate at the aroyl carbon.

To achieve H_2S production via Pathway A, the rate of hydrolysis (Pathway B) must be slow relative to the rate of H_2S release from reaction with cysteine. Hydrolysis of compounds **1a**–**1j** was evaluated using absorbance spectroscopy (Figure S6). In these experiments, the concentration of the SATOs relative to their hydrolysis products (SATHA and ketone or aldehyde) was determined over multiple half-lives. In general, SATOs hydrolyzed with half-lives in the time scale of days for compounds **1a**–**1j** excluding **1g** (Table 1).

The hydrolysis of SATOs followed first-order kinetics but did not conform to the expected linear free energy trend.[14] The unsubstituted compound (**1a**) hydrolyzed faster than the −F, −COOH, and $-OCH_3$ functionalized compounds (**1b**, **1c**, and **1d**). This type of behavior has been observed in other systems.[15] The ketone-derived SATOs hydrolyzed more slowly and showed a stability order of **1i** > **1h** > **1j**.

To evaluate our ability to control the rate of H_2S release from SATOs, we measured H_2S release using two different methods: (1) a microelectrode-based method that gives real-time data on H_2S concentration and (2) a colorimetric method that is better suited for longer release periods and was used to determine the half-life of H_2S release for specific compounds (Figure 1).

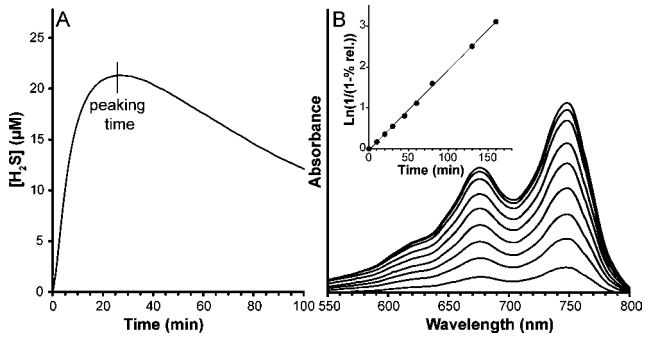


Figure 1. (A) Representative H_2S release curve of **1a** measured by the microelctrode method and (B) kinetics as measured by the methylene blue spectrophotometric method (10–200 min with intensity increasing over time). Inset shows the kinetics plot derived from absorbances measured at 676 nm. Experiments were conducted in triplicate.

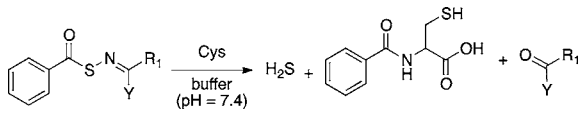
Additionally, the colorimetric method allowed for determination of H_2S release kinetics from compounds that were insoluble in the conditions required for the microelectrode experiments (**1l**–**1n**).

For the microelectrode experiments, SATOs **1a**–**1j**, **1k**, and **1o**–**1p** were dissolved in THF and added to buffered solutions containing 1 mM cysteine. Upon addition of the SATO, H_2S release was monitored continuously with an H_2S-selective microelectrode (Figure 1A). The H_2S concentration reached a maximum value, which is defined as the peaking time (Tables 2 and 3), after which it began to decrease as the result of volatilization and oxidation by O_2.[7]

In general, substitution at the *para* position of the arylaldehyde/ketone ring (**1a**–**1d**) has little effect on H_2S release rate (Table 2). Although the rate of H_2S release does not exhibit a strong dependence on the electronics of the substituent on the arylaldehyde ring, the rate of hydrolysis is influenced by substitution at this position. Taken together,

dx.doi.org/10.1021/ol500385a | Org. Lett. 2014, 16, 1558–1561

Table 2. H_2S Release from Substituted SATOs

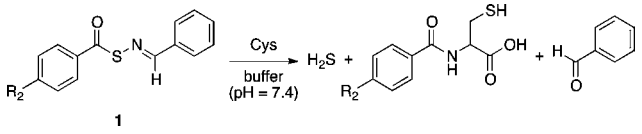


1

compd	peaking time (min)[a]	compd	peaking time (min)[a]
1a	24 ± 2	**1f**	26 ± 3
1b	20 ± 2	**1g**	27 ± 2
1c	18 ± 1	**1h**	34 ± 2
1d	22 ± 3	**1i**	34 ± 1
1e	14.8 ± 0.3	**1j**	33.5 ± 0.9

[a]Time after which [H_2S] reached a maximum value using an H_2S sensitive microelectrode. Studies were conducted at 40 μM SATO in 0.1% THF in PBS buffer (pH = 7.4) at rt in the presence of 1 mM cysteine.

Table 3. H_2S Release from Substituted SATOs



1

R_2	peaking time (min)[a]	$t_{1/2}$ H_2S release (min)[b]
H (**1a**)	24 ± 2	34 ± 6
F (**1k**)	21 ± 2	29 ± 2
Cl (**1l**)	–[c]	18 ± 1
CF_3 (**1m**)	–[c]	11 ± 1
CN (**1n**)	–[c]	8 ± 1
CH_3 (**1o**)	33.7 ± 0.4	52.9 ± 1.4
OCH_3 (**1p**)	36 ± 3	82 ± 11

[a]Time after which [H_2S] reached a maximum value detected by an H_2S sensitive microelectrode. Studies were conducted at 40 μM SATO in 0.1% THF in PBS buffer (pH = 7.4) at rt in the presence of 1 mM cysteine. [b]Half-lives of release were measured by using the methylene blue assay at 100 μM SATO in 30% THF in PBS buffer (pH = 7.4) at rt in the presence of 1 mM cysteine. [c]Samples were not soluble in the media for the microelectrode probe assay.

these data suggest that the stability of SATO*s* can be tuned without drastically altering the H_2S release profile.

We also tested several other molecules as triggers for H_2S release (Figure S2). Glutathione was shown to trigger H_2S release at levels similar to the case of cysteine, while *N*-acetylcysteine was found to trigger H_2S generation at a slower rate than cysteine. However, neither lysine nor serine triggered H_2S release, nor was H_2S generation observed in aqueous solutions of SATO in the absence of thiol functionality. Additionally, H_2S release was evaluated at different cysteine concentrations. In general, higher concentrations of cysteine resulted in an increased H_2S release rate (Figure S3). H_2S generation was also observed in plasma (Figure S5).

The colorimetric method of measuring the concentration of H_2S involves the H_2S-promoted conversion of *N*,*N*-dimethyl-*p*-phenylenediamine into methylene blue,[16] which can be measured by its peak absorbance at 676 nm (Figure 1B). As real-time detection is not possible with the methylene blue method, time points are taken at various intervals for each compound. We note that the methylene blue method can overestimate H_2S levels, so we make no attempt here to translate our measurements to actual H_2S concentrations.[17]

Half-lives for the pseudo-first-order kinetic plots were determined (Figure 1B inset) and found to be on the order of 8–82 min. In general, results were similar to the electrochemical method, with H_2S release half-lives trending based on SATHA ring substituent electronics. A Hammett plot was constructed to quantify the effect of substituents on the rate of H_2S release (Figure 2). The Hammett plot revealed a

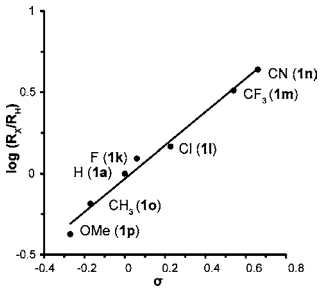
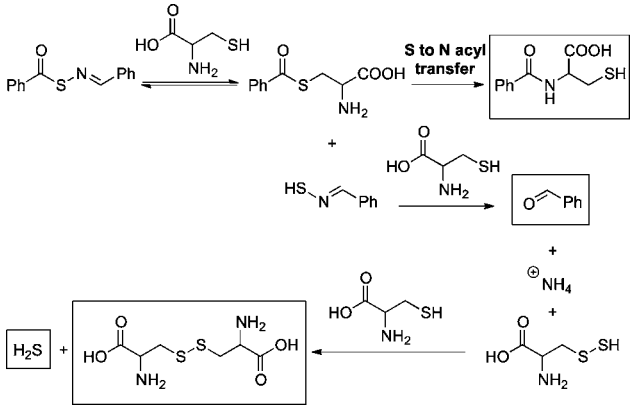


Figure 2. Hammett plot of the rate of H_2S release relative to **1a** vs the σ value of the *p*-substituent (ρ = 1.05, R^2 = 0.986).

strong dependence of the rate of H_2S release on the σ values of the SATHA substituents (ρ = 1.05). It follows from Figure 2 that the H_2S release kinetics of SATOs can be finely tuned. The strong correlation between H_2S release rate and σ suggests that the rate of H_2S release for other SATOs could be predicted from the Hammett plot. These data represent an unprecedented level of control over the rate of H_2S release.

The fast reaction between SATOs and cysteine to generate H_2S ($t_{1/2}$ ≈ 8–82 min) compared with their hydrolysis rate ($t_{1/2}$ ≈ 45–250 h) rules out pathway B as the operative H_2S release mechanism for most SATOs under the conditions tested. To study the mechanism of H_2S release, the products of the reaction between **1a** and cysteine were analyzed. Based on the compounds isolated by HPLC, we propose that H_2S may be generated according to Scheme 3. First, reversible thiol

Scheme 3. Proposed Mechanism of H_2S Release[a]



[a]Boxed products were either isolated from the reaction mixture or detected indirectly.

exchange between the SATO and cysteine gives the arylidenethiooxime along with *N*-benzoylated cysteine after a S → N acyl transfer step. Reaction of a second equivalent of cysteine with the arylidenethiooxime generates thiocysteine, ammonia, and the original aldehyde. Thiocysteine then reacts with another equivalent of cysteine to generate cystine and H_2S.[18] Further mechanistic work is needed to evaluate whether this is the

dx.doi.org/10.1021/ol500385a | *Org. Lett.* 2014, 16, 1558–1561

operative mechanism for H_2S release. See the Supporting Information for further discussion.

Regardless of the pathway of H_2S release, the parent aldehyde/ketone is regenerated in the process. This implies that SATHA can be conjugated to aldehyde- or ketone-bearing therapeutic agents such as cinnamaldehyde (as in **1f**), known to possess antimicrobial[19] and anticancer[20] properties, to impart tandem physiological activity. However, the requirement for a ketone or aldehyde limits the types of drugs amenable to this type of derivatization to those bearing these functionalities. H_2S donor-drug hybrids such as NOSH-aspirin[8a] and H_2S-releasing derivatives of diclofenac[9] have been reported and in many cases show improved physiological responses compared with their parent drugs.

In summary, we have synthesized a series of SATOs from SATHAs and common aldehydes and ketones. We showed that SATOs are relatively stable in aqueous solution at physiological pH, and hydrolysis of these compounds can be controlled to a degree by tuning the steric and electronic factors of the SATO bond. We demonstrated that the half-life of H_2S release could be varied between 8 and 82 min simply by changing the substituent on the SATHA ring. Given the ease of installation of this versatile functional group, we envision that SATOs could open new avenues for the study of H_2S biology and could enable a new generation of H_2S-releasing therapeutics and H_2S-drug conjugates.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic details, characterization data, and kinetics plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Institute for Critical Technology and Applied Science (JFC12-256). We thank Prof. Richard D. Gandour (Virginia Tech) for helpful discussions and Prof. Amanda J. Morris (Virginia Tech) and Prof. Tijana Z. Grove (Virginia Tech) for use of instruments.

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