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Esterase Sensitive Self-Immolative Sulfur Dioxide Donors

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Supporting Information

ABSTRACT: A series of cell-permeable esterase-sensitive sulfonates that undergo self-immolation to produce sulfur dioxide (SO_2), a gaseous pollutant with new and emerging biological roles, is reported. These compounds should facilitate the study SO_2 biology and will lay the platform for newer stimuli-responsive donors of this gas.

Gaseous signaling molecules derived from nitrogen, carbon, and sulfur are ubiquitous in nature and are emerging as major modulators of diverse physiological processes.[1] For example, hydrogen sulfide (H_2S) has numerous roles in neuromodulation, pathogen response to antibiotics,[2] and blood vessel relaxation. Sulfur dioxide (SO_2),[3] a potential product of oxidation of H_2S, similarly has recently assumed importance as a mediator of cellular processes.[4] SO_2 is widely used in the food industry as a preservative and as an antibacterial agent.[5] At diminished concentrations, SO_2 can mediate signaling such as vasodilation.[6] However, at elevated concentrations, SO_2 can damage biomacromolecules such as DNA, especially in the presence of metal ions.[7−9] Thus, controlled generation of this gaseous molecule has enormous potential but is challenging.

Biochemical and cellular experiments carried out with gaseous SO_2 are somewhat unreliable, associated with potential variations, and have limited therapeutic potential. The most commonly used inorganic donor of sulfite, which is a mixture of sulfite and bisulfite has limited permeability and is thus used in high millimolar concentrations.[5,6] These elevated concentrations may compromise appropriate interpretations regarding the effects of SO_2. Thus, small molecule donors of this gas assume importance.[10] Our laboratory reported benzosultines as donors of SO_2 under ambient physiological pH conditions (Figure 1).[11] These donors spontaneously undergo retro Diels−Alder to generate SO_2 with moderately tunable rates. Binghe Wang and co-workers have recently shown a "click and release" strategy for generation of sulfur dioxide (Figure 1).[12,13] These donors have the unique advantage of having a wide range of rates of SO_2 generation. However, these donors are more akin to spontaneous donors of

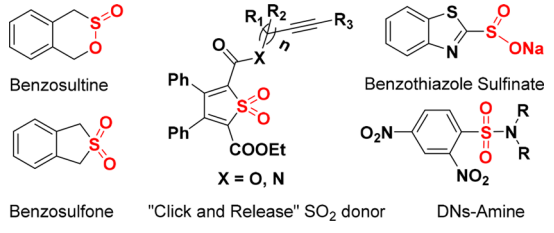

Figure 1. Representative examples of sulfur dioxide donors.

SO_2 and again, incorporation of a trigger will help in controlling delivery of this gas. Xian and co-workers have recently reported a sulfinate which undergoes hydrolysis to produce SO_2.[14] They have also shown the vasodilatory effects of this molecule underscoring the importance of controlled generation of SO_2. However, this donor does not contain a physiological trigger and this may be a limitation for further exploitation of therapeutic potential of this gas.

Photolabile SO_2 donors have also been reported. Benzosulfones were found to be photolabile under ultraviolet irradiation conditions to produce SO_2.[15] A similar class of phototriggerable SO_2 donors were reported by Uchida and co-workers.[16] The use of ultraviolet light, however, has limited utility and may be harmful to cells due to phototoxicity. Our laboratory has reported 2,4-dinitrophenylsulfonamides (DNs-Amine) as thiol-activated sulfur dioxide donors (Figure 1).[17−19] While these donors have triggerable SO_2 donation, the use of thiols, which are a major antioxidant within cells, as triggers may not be useful to study SO_2, a redox-active gaseous species. It is thus desirable to have a relatively innocuous stimulus such as esterase (ES) to trigger SO_2 generation within cells.

In order to design a new class of esterase-sensitive SO_2 donors, we considered the chemistry associated with decomposition of carbonates, which have been extensively used for drug delivery and they operate by generation of carbon dioxide (CO_2), which presumably is an irreversible reaction. An appropriately placed trigger and subsequent self-immolation can produce CO_2 from a carbonate (Scheme 1a). The key bond that breaks is the C−O bond shown by an arrow, whose estimated bond dissociation energy (BDE) is 68 kcal·mol^{-1}. Similarly, it was envisaged that cleavage of a C−S bond in sulfonates (shown by an arrow) will similarly trigger generation of SO_2 and an alcohol. The BDE of this bond is estimated to be 57 kcal·mol^{-1}, which is comparable with the C−O bond.[20] If successful, such a method may have broad relevance as a probe for SO_2 biology as well as a methodology for codelivery of an alcohol-based drug. Further-

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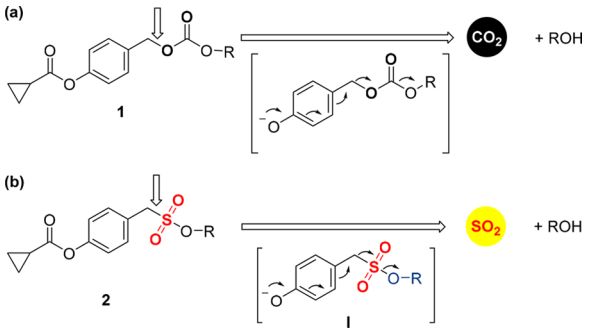
Scheme 1. (a) Esterase-Cleavable Carbonate Should Generate CO_2 and an Alcohol (1a, R = Umbelliferone (Figure 2)); (b) Proposed Esterase Cleavable Sulfonates That Are Expected To Generate SO_2 and an Alcohol



more, the nature of the leaving group i.e. the alcohol may determine the SO_2 generation capability.

In order to test this hypothesis, first, the fluorophore umbelliferone (Umb, Figure 2) was derivatized. The carbonate

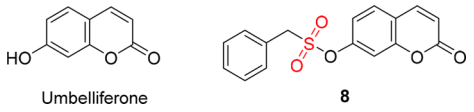
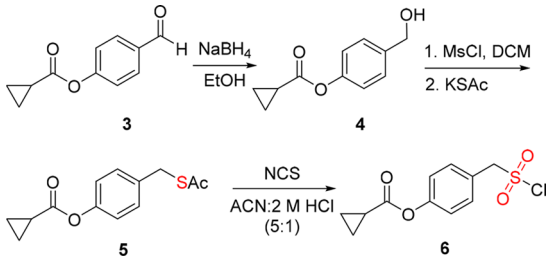


Figure 2. Structures of umbelliferone and **8**.

1a (Scheme 1a) was synthesized (see the SI). Due to the improved hydrolytic stability of cyclopropyl esters when compared with other aliphatic esters, this ester was chosen.[21,22] The pivaloyloxymethyl group was not considered as sulfite is known to react with formaldehyde, a byproduct of decomposition.[23] In order to synthesize the sulfonate **2a**, first, the aldehyde **3** was synthesized from 4-hydroxybenzaldehyde (Scheme 2) Reduction with sodium borohydride gave **4**, which

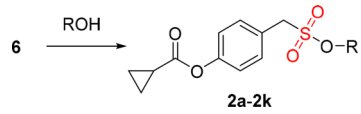
Scheme 2. Synthesis of the Sulfonyl Chloride 6



was then converted to the thioacetate **5** in two steps. The thioacetate was treated with *N*-chlorosuccinamide to afford the sulfonyl chloride **6**.[24] Treatment of **6** with umbelliferone (Figure 2) gave **2a** (Table 1). The structure of **2a** was confirmed by X-ray diffraction analysis of crystalline material (see Figure S1).

The carbonate **1a** and the sulfonate **2a** were independently treated with esterase.[22,25] HPLC analysis of the reaction mixture after 10 min revealed complete disappearance of the **1a** (see Figure S2). The disappearance of **2a** (Figure 3a) was somewhat slower when compared with **1a** (0.54 ± 0.08 min^{-1}). The rate constant was found to be 0.13 min^{-1} and the half-life was 5 min. This rate is comparable with the formation of umbelliferone, 0.18 min^{-1} monitored under similar conditions (Figure 3b). The yield

Table 1. Synthesis of Sulfonates 2a−k



R	pK_a of ROH[a]	product	yield (%)
umbelliferone	7.7	**2a**	50
Ph	9.9	**2b**	75
4-OMePh	10.2	**2c**	65
4-NO_2Ph	7.1	**2d**	70
(2-*tert*-b	10.2	**2e**	65
(3,5-dimethyl-4-Cl)Ph	9.4	**2f**	72
Bn	15.4	**2g**	23
allyl	15.5	**2h**	27
propargyl	13.6	**2i**	24
n-butyl	16.0	**2j**	53
*i*Pr	17.0	**2k**	44

[a]Values are from literature and are either for the compound itself or analogues with similar structures. References 27−31.

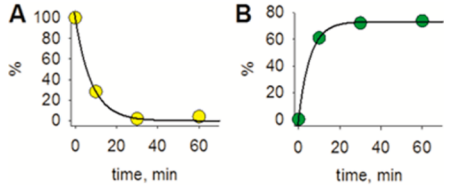
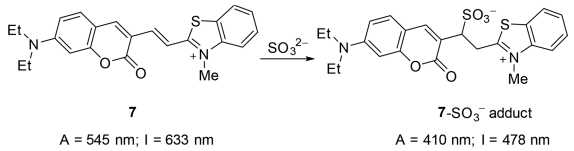


Figure 3. (a) HPLC analysis of **2a**. Compound **2a** (50 μM) in PBS (pH 7.4, 10 mM) at 37 °C was incubated in the presence of esterase showed the disappearance of **2a**. Rate constant for disappearance was found as 0.13 ± 0.01 min^{-1}. (b) HPLC analysis of the reaction mixture for Umb. Rate constant for appearance of Umb was found as 0.18 ± 0.006 min^{-1} (for a detailed protocol, see the SI).

of Umb was 80% suggesting an efficient conversion of **2a** to Umb in the prescence of esterase.

A coumarin−hemicyanine dye **7** (Scheme 3),[26] for colorimetric as well as fluorescence-based detection of sulfite (SO_3^{2-}), the

Scheme 3. Dye 7 and the Adduct It Forms upon Reaction with Sulfite



hydrated form of SO_2, was synthesized using reported procedures. This probe has been found to be useful in in vitro as well as cellular studies to detect SO_2. The dye **7** reacts with sulfite to produce a covalent adduct (Scheme 3). This results in a characteristic decrease in absorbance at 545 nm with concomitant increase in the absorbance signal at 410 nm (Figure 4a). In the presence of **2a** and esterase, a decrease in the absorbance at 545 nm was seen (Figure 4b). Due to the significant absorbance of Umb at 410 nm, the use of **7** to accurately determine sulfite is not possible. However, based on the diminution of the absorbance at 545 nm, the generation of sulfite during decomposition of **2a** in the presence of esterase was inferred (see Figure S6).

In the absence of esterase, no significant decomposition of **1a** or **2a** was observed (see Figure S3), suggesting that the cyclopropylester is not susceptible to hydrolysis. When **2a** was

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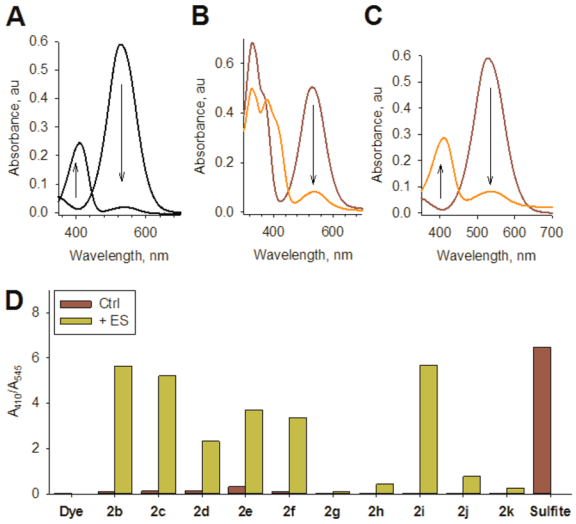


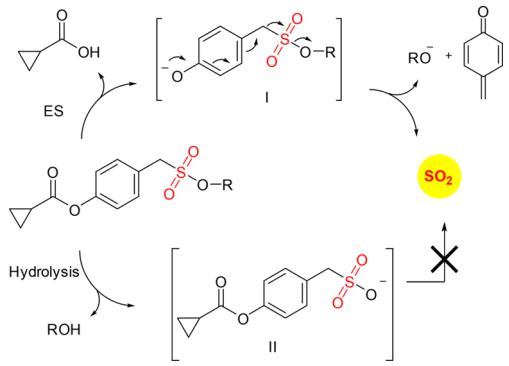
Figure 4. (a) Incubation of **7** (10 μM) in the presence of $NaHSO_3$ (50 μM) for 15 min shows a decrease in absorbance at 545 nm and a corresponding increase in absorbance at 410 nm.; (b) Incubation of **2a** + ES in the presence of **7** (10 μM) for 15 min. Disappearance of the 545 nm signal was observed and is indicative of sulfite formation. Concentration of **2a** was 50 μM. This experiment was carried out in the presence of Umb (see Figure S6). (c) Incubation of **7** (10 μM) in the presence of **2e** + ES for 15 min shows an absorbance profile comparable with $NaHSO_3$. (d) Ratio of absorbance at 410 nm (A_{410}) to the absorbance at 545 nm (A_{545}) during incubation of **7** with various compounds (Ctrl) and in the presence of ES (see Figure S7). Concentration of **2e** was 50 μM. All of the experiments were conducted in PBS (pH 7.4, 10 mM) at 37 °C (for a detailed protocol, see the SI).

pretreated with an esterase inhibitor (phenylmethanesulfonyl fluoride, PMSF)[32] and subsequent exposure to ES, a diminished signal for Umb was observed (see Figure S4). Preincubation of ES with PMSF and subsequent addition of **2a** showed a dose-dependent decrease in fluorescence, again supporting catalysis by esterase is necessary for activation of **2a** (see Figure S4, inset). In the presence of common biological nucleophiles, **2a** was found to be stable (see Figure S4). In basic pH (9.2), significant formation of Umb from the hydrolysis of the carbonate was observed; while the sulfonate remained stable. This enhanced stability of the sulfonate may present opportunities for delivering SO_2-based hybrid drugs.

In order to study the reactivity of the sulfonate functional group toward esterase, compound **8** (Figure 2) was synthesized. When treated with esterase, no significant fluorescence signal corresponding to Umb formation was recorded (see Figure S4). A similar result was observed in human cervical cancer HeLa cell lysate during 1 h suggesting that the sulfonate group is stable under cellular conditions (see Figure S5). Incubation of **2a**, as expected, produced a fluorescence signal confirming the production of Umb. Taken together, our data supports the selectivity of cleavage by esterase and the broad-range stability of the sulfonate group to cellular nucleophiles.

Having established that **2a** was capable of undergoing self-immolation in the presence of esterase to generate SO_2, we next proceeded to study the effect of the leaving group on this process. A series of aromatic alcohols were independently reacted with **6** to produce the corresponding sulfonates **2b**–**f** (Table 1). Again, the SO_2-sensitive dye **7** was used to assess the capability of these compounds to generate SO_2. These compounds were next exposed to esterase in the presence of the dye **7**. A representative absorbance profile for **2e** is shown in Figure 4c. The ratio of absorbance at 410 nm (A_{410}) to the absorbance at 545 nm (A_{545}) provides an estimate of the ability of the compound to produce SO_2. Colorimetric analysis revealed that all the aforementioned compounds were capable of generating sulfite. The dye **7** is also used as a sulfite-sensitive fluorescence probe: the free probe displayed a red emission with the maximum at 633 nm (excitation at 545 nm). In the presence of sulfite, the fluorescence signal at this wavelength is diminished with a concomitant increase in a new blue emission peak at 478 nm (excitation at 410 nm). I_{478}/I_{633} is hence a measure of SO_2 donating capability. The fluorescence data corroborated the absorbance data that was obtained with the donors. Together, our data supports a mechanism of ester hydrolysis leading to formation of intermediate I, which undergoes self-immolation to produce an alkoxide and a quinone methide (Scheme 4).

Scheme 4. Mechanism of Generation of SO_2 from Sulfonates[a]



[a]For sulfonates derived from aromatic alcohols, SO_2 generation is dominant. Certain sulfonates derived from aliphatic alcohols were susceptible to hydrolysis (through II) and were poor SO_2 donors or are possibly trapped as intermediate I.

Next, sulfonates **2g**–**k**, which are derived from aliphatic alcohols were synthesized. When estimated for SO_2 in the presence of esterase, **2g**, **2h**, and **2k** did not show a significant shift in the absorbance profile, and accordingly, the ratio of A_{410}/A_{545} was diminished (Figure 4d). This data suggests that these compounds are poor donors of SO_2 when compared with their counterparts. Being aliphatic alcohols (Scheme 4), their leaving group ability is significantly lower than aromatic alcohols and this may contribute to diminished yield (see Table 1 for pK_a values). Prolonged incubation in buffer of these reaction mixtures also did not produce significant levels of sulfite. Since the cyclopropyl ester is stable toward hydrolysis in pH 7.4 buffer, the propensity for the sulfonate group to undergo hydrolysis to produce II was next examined. Compounds **2g**–**k** were independently incubated in pH 7.4 buffer, and we found that all compounds except for the benzyl and allyl compounds were stable toward hydrolysis (see Figure S12). When the benzyl derivative was incubated in buffer, nearly complete disappearance in 30 min was seen. While partial hydrolysis was observed in the case of the allyl derivative, this data suggested that certain sulfonates were susceptible to hydrolysis. Thus, among aliphatic alcohol-derived sulfonates, SO_2 generation after activation by esterase is possible provided the sulfonate is not susceptible to hydrolysis and if the alcohol was a good leaving group (such as propargyl alcohol).

Next, we investigated the capability of the SO_2 donors to permeate cells and generate SO_2. Accordingly, the dye **7** was used

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in A549 lung carcinoma cells. In the presence of sulfite (200 μM), a distinct increase in the signal in the blue channel with concomitant decrease in signal in the red channel was observed (Figure 5 and Figure S13). The SO_2 donors **2c** was similarly found

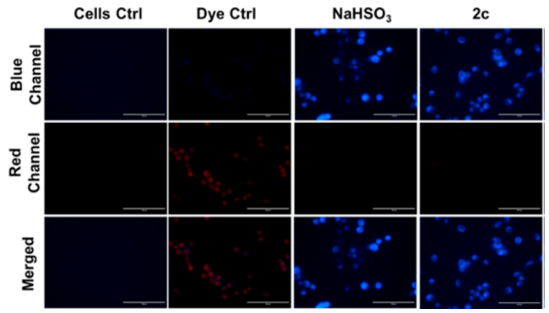

Figure 5. Fluorescence microscopy images of A549 cells treated with dye for 30 min and followed by treatment of the compound for 30 min in PBS (pH 7.4, 10 mM) at 37 °C. Fluorescence signal was monitored in red channel (7) and the blue channel (7-SO_3^- adduct). Final concentrations of dye 7 was 10 μM; $NaHSO_3$, 200 μM; sulfonates, 25 μM. Scale bar = 200 μm (for a detailed protocol, see the SI).

to fluoresce in the blue channel but at a much lower concentration (25 μM) suggesting their superior capability to generate sulfite within cells. Compounds **2g** and **2h**, which were poor SO_2 donors in the presence of esterase were similarly found to be incapable of generating SO_2 within cells. Lastly, the cytotoxicity of these compounds was estimated using two different cell lines and a majority of these compounds were not significantly cytotoxic (see Figure S14 for A549 cells and Figure S15 for HeLa cells). The likely byproducts that were produced during decomposition of SO_2 donors **2c** and **2i** were not cytotoxic either (see Figure S16). Thus, the SO_2 donors prepared in this study are cell-permeable and appear to be well tolerated by cells and should facilitate a better understanding of the biology of this gas.[33]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02544.

Synthesis, characterization data, protocols for assays, and X-ray data for **2a** (PDF)

Accession Codes

CCDC 1585422 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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