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Benzosultines as Sulfur Dioxide (SO_2) Donors

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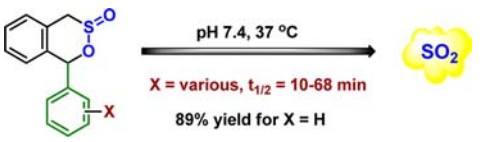
ABSTRACT



In order to understand precise biological roles of sulfur dioxide (SO_2), reliable SO_2 donors, compounds that produce SO_2 under physiological conditions, are necessary. The design and development of 1-phenyl-benzosultine as an efficient SO_2 donor is reported. This compound undergoes cycloreversion to generate SO_2 upon dissolution in aqueous buffer at 37 °C with a yield of 89% and a half-life of 39 min and shows SO_2-like biological activity in a DNA cleavage assay.

Sulfur dioxide (SO_2), a byproduct of combustion of fossil fuels, volcanic emissions, and industrial processes, is a toxic environmental pollutant with a profound impact on habitat suitability for plant as well as animal life.[1] Human exposure to SO_2 occurs through inhalation of gaseous SO_2 and through food and cosmetics. Its hydrated forms bisulfite, HSO_3^-, and sulfite, SO_3^{2-}, find routine use as antibiotics, preservatives, and antioxidants.[2] SO_2 is also produced in cells during oxidation of hydrogen sulfide (H_2S) and decomposition of β-sulfinylpyruvate to pyruvate.[3] Some recent evidence supports potential regulatory roles for this gas in the cardiovascular system including during hypoxia and inflammatory responses.

Despite the importance of SO_2 for living systems, the molecular mechanisms of SO_2 biology remain poorly understood.[3] Because SO_2 is a reactive gas, obtaining biological data with it is cumbersome. For mechanistic studies, typically either gaseous SO_2 or a mixture of sulfite and bisulfite is utilized as a surrogate.[1] While useful, such exogenous sources lack temporal control over SO_2 generation and, thus, may not be effective in mimicking endogenously produced SO_2. Recently, 2,4-dinitrophenylsulfonamides were reported as a class of thiol-mediated SO_2 donors.[4] However, the use of thiol as a trigger in 2,4-dinitrophenylsulfonamides may complicate mechanistic studies because targets of SO_2 include biologically relevant disulfides and thiols.[3] Thus, organic compounds that can be used for controlled generation of SO_2 under nonenzymatic and physiological conditions are not yet available.

1,4-Dihydro-2,3-benzoxathiin-3-oxides (benzosultines) offer the potential for efficient generation of SO_2 through a thermal retro-Diels–Alder reaction (Table 1). Perturbation of sterics and electronics of the incipient 1,3-diene (such as **1b**) might provide opportunities for tuning SO_2 release profiles from benzosultines (such as **1a**). Herein, we have presented the results of our work toward the development of a new series of benzosultines as sulfur dioxide donors with potential for use as tools in biochemical studies.

Previous reports have shown that benzosultines such as **1a** are stable up to 80 °C.[5] In order for benzosultines to be useful as organic SO_2 donors, their cycloreversion must occur at physiological temperature of 37 °C. Clearly, lower

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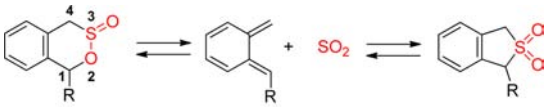
Table 1. Calculated Thermodynamic and Kinetic Data at 37 °C in kcal/mol for Conversion of Benzosultines **1a**–**3a** or Sulfones **1c**–**3c** To Produce 1,3-Dienes **1b**–**3b** Respectively and Sulfur Dioxide[a]



reactant species	ΔE_{rxn}[b]	ΔG_{rxn}[b]	$\Delta G^{\ddagger}$[c]	$\Delta\Delta G^{\ddagger}$[d]
boat-ax-**1a**	**33.9**	**15.3**	**29.5**	**0**
boat-eq-**1a**	35.4	16.4	31.0	1.5
1c	37.6	18.3	33.5	4.0
cis-boat-ax-**2a**	**36.3**	**16.6**	**28.1**	**−1.4**
trans-boat-eq-**2a**	38.2	18.4	30.4	−0.9
cis-**2c**	39.8	19.6	32.9	3.4
cis-boat-ax- **3a**	**34.5**	**15.9**	**26.3**	**−3.2**
trans-boat-eq-**3a**	34.6	16.3	27.9	−1.5
cis-**3c**	37.6	18.6	29.5	0

[a] The data are for two lowest barrier pathways for benzosultines and the lowest barrier pathway for sulfones. Energy values in bold correspond to the most favorable pathway (lowest activation barrier) for the cycloreversion of **1a**–**3a**. [b] The significant difference between ΔE_{rxn} and ΔG_{rxn} is because of the increase in the number of molecular species during the cycloreversion leading to a large translational and rotational entropy contribution to the driving force of the reaction. [c] Vibrational entropy and zero-point energy contributions lower the reaction barriers as compared to the pure electronic barrier (see the Supporting Information, Tables S1–S3). This is because of the relatively floppy nature of the transition state with respect to the reactant, which leads to a lower zero-point energy and higher vibrational entropy. [d] Calculated by using boat-ax-**1a** as the reference.

cycloreversion barriers as compared to **1a** are desired, and the literature suggests that a way to achieve this is by suitable substitution on the carbon atom adjacent to oxygen in the sultine ring.[6] Keeping this design principle in mind, we calculated the Gibbs free energy barriers for the cycloreversion of the unsubstituted and substituted benzosultines **1a**, **2a**, and **3a** to the corresponding dienes **1b**, **2b**, and **3b**, and SO_2 using *ab initio* theoretical methods. The optimized structures, energies, vibrational frequencies, and intrinsic reaction coordinates (IRCs) were all calculated at the Møller–Plesset second-order perturbation (MP2) level of theory with the 6-31G(d,p) basis set. All calculations were performed using the GAMESS program package.[7]

Compound **1a** was found to have four stable conformations (Figure 1). In two of the conformers, the six-membered sultine ring is in the pseudochair conformation, while, in the two others, the sultine ring is in the pseudoboat conformation. The notation "ax" (axial) or "eq" (equatorial) in the figure labels refers to the orientation of the S=O bond with respect to the plane of the ring. The methyl and phenyl derivaties **2a** and **3a** were found to have seven stable diastereomers each (see the Supporting Information (SI), Figures S1 and S2). The greater number of isomers in these cases as compared to **1a** is due to the two different orientations of the −R group attached to the carbon atom next to the oxygen. The prefix *cis* or *trans* is used to differentiate the relative orientation of −R with respect to the S=O bond. For a particular −R, the individual *cis* isomers can interconvert among themselves and so can the *trans* isomers, but the *cis* isomers cannot convert to the *trans* isomers and vice versa without cycloreversion to the 1,3-diene and SO_2. In all cases, **1a**, **2a**, and **3a**, IRC calculations and comparison of Gibbs free energy values of the transition state (TS) structures suggest that two of the stereoisomers, namely the *cis*-boat-ax (boat-ax in case of **1a**) and *trans*-boat-eq (boat-eq in case of **1a**) stereoisomers have significantly lower barriers for cycloreversion than the other isomers and are the only ones likely to be mechanistically relevant. These involve the *endo* or *exo* stereoisomers of the TS structures rather than planar TS structures (see the SI), which correspond to higher energy pathways. Moreover, the diene formed on cycloreversion of the sultine is in the (*E*) form, consistent with prior studies of hetero-Diels–Alder reactions.[6] The structures of the *cis*-boat-ax and *trans*-boat-eq sultines, their cycloreversion products, and corresponding TSs for R = Ph are shown in Figure 2. The reaction energies (ΔE_{rxn}), Gibbs free energies (ΔG_{rxn}), and Gibbs free-energy barriers ($\Delta G^{\ddagger}$) of the mechanistically relevant species calculated at 37 °C are presented in Table 1.

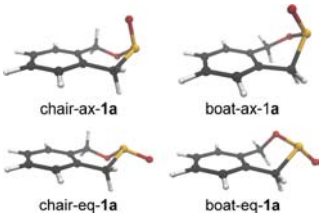


Figure 1. Stable conformations of **1a**.

The most favorable path for cycloreversion of the benzosultines is through the *endo* (*E*) TS starting from the *cis*-boat-ax stereoisomers. For this stereoisomer, comparing across **1a**, **2a**, and **3a**, we can see that methyl substitution (**2a**) reduces the cycloreversion barrier by 1.4 kcal/mol possibly due to the electron-donating effect of a methyl group, while phenyl substitution (**3a**) reduces the barrier by 3.2 kcal/mol possibly due to extended conjugation (Table 1). At 37 °C, this barrier reduction corresponds to an ~10-fold rate enhancement in the rate of SO_2 generation in the case of methyl substitution (**2a**) and an ~200-fold rate enhancement in the case of phenyl substitution (**3a**) as compared to **1a**.

1,3-Dienes such as **1b** undergo cheletropic addition of SO_2 to produce a sulfone (**1c**). As in the case of sultines, calculations of **1c**–**3c** were perfomed (Table 1 and Figure 2; see SI Figures S3–S5). From IRC calculations, we found

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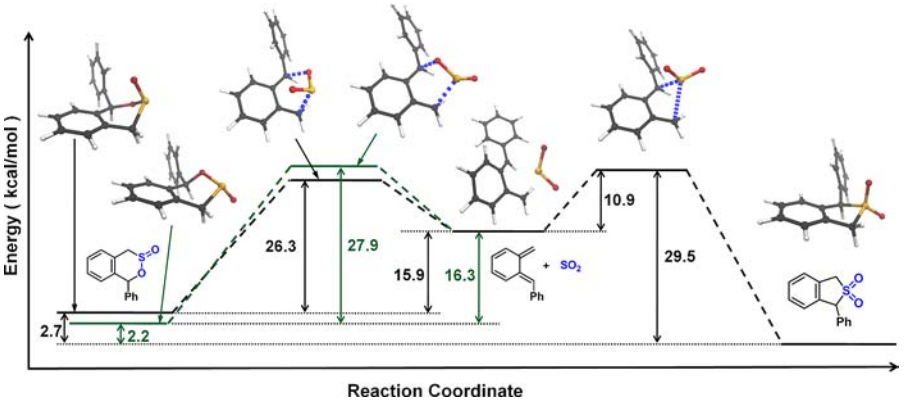


Figure 2. Gibbs free energy profiles for decomposition of **3a** to produce **3b** and SO_2 and its conversion to **3c** at 37 °C.

only one mechanistically relevant pathway for sulfone formation in all cases. The corresponding product has the SO_2 moiety and the −R group both on the same side of the five-membered ring and is therefore referred to as *cis*. The sulfones **1c**−**3c** are found to be both thermodynamically and kinetically more stable than the corresponding sultines (Table 1). The 1,3-diene and SO_2, once formed, can react to form either the sultine or the sulfone: the $\Delta G^{\ddagger}$ for formation of **3a** or **3c** from **3b** is sufficiently low (< 11 kcal/mol) that both reactions occur easily at 37 °C (Figure 2). However, once formed, the $\Delta G^{\ddagger}$ for extrusion of SO_2 from **3c** is relatively large (29.5 kcal/mol) and the reaction would be ~200 times slower than the cycloreversion of **3a**. We thus predict that **3a** eventually completely converts to **3c**.[5]

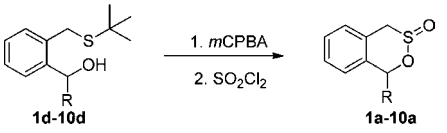
Benzosultines **1a**−**3a** were synthesized in several steps from commercially available phthalide (see the SI) with a sulfuryl chloride mediated cyclization reaction as the final step (Table 2, entries 1−3).

HPLC analysis of acetonitrile solutions of **1a**−**3a** at 70 °C for 3 h showed no significant decomposition of **1a** (< 5%), whereas, during this time, **2a** was 41% decomposed while **3a** was completely decomposed in this time period (see the SI, Table S5). These results are in agreement with the predicted barriers for cycloreversion being **3a** > **2a** > **1a** (Table 1) and similar to a previous report of rates of reactions of **1a**−**3a** at 80 °C being **3a** > **2a** > **1a**.[5]

When a similar decomposition experiment was conducted at a physiological temperature of 37 °C in MeCN, **3a** decomposed ($k = 2.9 \times 10^{-3}$ min^{-1}) to produce the sulfone **3c** (4.5×10^{-3} min^{-1}) in 92% yield (see the SI, Figure S6). No significant differences in the rates of decomposition of the major or minor diastereomers of **3a** were found suggesting that both *cis* and *trans* isomers decompose at comparable rates (see the SI, Figure S6). Under similar conditions, sultines **1a** and **2a** were found to be unreactive. No significant decomposition of the sulfone **3c** at 37 °C was observed supporting the predicted mechanism that the formation of **3c** from **3a** was an irreversible process.

At physiological pH 7.4 and 37 °C, **3a** was nearly completely decomposed with a rate constant $k = 0.0140$ min^{-1} (Figure 3).[8] The major inorganic product of this decomposition was SO_2, measured as sulfite (89% yield) with a half-life of SO_2 generation of 39 min (Table 3).

Table 2. Synthesis of **1a**−**10a**



entry	R	compd	product	yield (%)
1	H	**1d**	**1a**	60
2	Me	**2d**	**2a**	88
3	Ph	**3d**	**3a**	43[a]
4	4-FPh	**4d**	**4a**	42
5	4-ClPh	**5d**	**5a**	49
6	4-CNPh	**6d**	**6a**	51
7	4-MePh	**7d**	**7a**	38
8	3-OMePh	**8d**	**8a**	32
9	3-CNPh	**9d**	**9a**	38
10	2-NO_2Ph	**10d**	**10a**	35

[a] ^{1}H NMR (12% minor) and HPLC (19% minor) analysis showed **3a** to be a mixture of *cis* and *trans* isomers. Computational studies identify the most stable *cis* isomer as *cis*-boat-ax-**3a** and the two lowest energy interconvertible *trans* isomers as boat *trans*-boat-eq-**3a** and *trans*-chair-ax-**3a** (see the SI, Tables S1−S3).

The suitability of **3a** for use as a SO_2 donor under biological assay conditions was demonstrated using a pBR322 supercoiled plasmid cleavage assay (Figure 4).[9] A nearly identical DNA damage profile in the presence of equimolar amounts of Cu(II) and **3a** or sulfites (1:3 Na_2SO_3 and $NaHSO_3$) was recorded (Figure 4). The DNA damage experiment was

(8) Stabilization of the activated complex by H-bonding was reported to be responsible for acceleration of the retro-Diels−Alder of anthracenedione in water in comparison with an aprotic organic solvent. A similar H-bonding effect might explain the observed increased rate of cycloreversion of **3a** in aqueous media. Wijnen, J. W.; Engberts, J. B. F. N. *J. Org. Chem.* **1997**, *62*, 2039.

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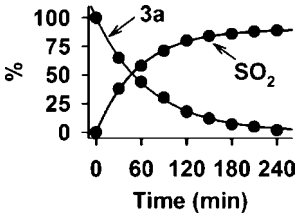

Figure 3. Decomposition of **3a** in pH 7.4 buffer at 37 °C produced SO_2.

conducted in two independent batches of **3a**, each in duplicate, and we found similar results in all cases demonstrating batch-to-batch reproducibility in the use of **3a** as a SO_2 donor.

Table 3. Decomposition Profiles of Benzosultines **3a**–**10a** Prepared in This Study in pH 7.4 Buffer at 37 °C and Substituent Constants used for Construction of Hammett Plot

compd	k (min^{-1})[a]	$t_{1/2}$ (min)[b]	max SO_2 yield (%)[c]	σ[d]	σ^+[e]
3a	0.0140	39	89	0	0
4a	0.0244	14	83	0.15	−0.073
5a	0.0140	34	79	0.24	0.114
6a	0.0093	38	76	0.71	0.659
7a	0.0336	13	80	−0.14	−0.311
8a	0.0104	56	73	0.12	0.047
9a	0.0069	68	59[f]	0.62	0.562
10a	0.0500	10	81	–	–

[a] Determined by kinetic analysis of decomposition of sultine using HPLC. All rate data obtained are for diastereomers where applicable. [b] Determined kinetic analysis of formation of SO_2 as sulfite by an ion chromatograph attached with a conductivity detector. [c] The highest yield of SO_2 produced after complete decomposition of sultine. [d] Reference 10a. [e] Reference 10b. [f] As no evidence for the formation of the corresponding sulfone was found, the diminished yields of SO_2 must be due to collateral consumption of the sultine through other yet uncharacterized processes.

1-Aryl-benzosultines also offer scope for tuning rates of SO_2 generation through systematic structural modifications on the 1-aryl ring. Using a methodology similar to that for the synthesis of **3a**, we prepared 1-aryl-benzosultines **4a**–**10a** (Table 2) with electron-donating and -withdrawing groups on the 1-aryl ring (see the SI, Table S4). All the sultines tested were found to be labile in pH 7.4 buffer at 37 °C, and half-lives of SO_2 generation varied from 10 to 68 min (Table 3). The maximum SO_2 yields were in excess of 75% in the majority of the sultines tested (Table 3).

A nearly linear Hammett plot constructed using rate constants for decomposition of **3a**–**9a** (Table 3) and substituent constant σ^+ suggested a predictable mechanism of decomposition for this scaffold (Figure 5).[10,11] The slope, ρ, was found to be −0.6 implying a weak electronic effect on the reaction center wherein an electron-donating group accelerated the cycloreversion.[12]

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(11) A nearly identical ρ-value of −0.6 (R^2 = 0.60) was obtained when σ was used to construct the Hammett plot.

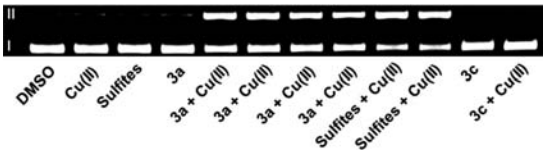

Figure 4. Nuclease activity of (100 μM of analyte) was determined by a pBR322 plasmid DNA (100 ng of form I DNA) cleavage assay in pH 8.0 buffer, incubated at 37 °C for 5 h. An aqueous solution of Na_2SO_3 and $NaHSO_3$ in a 1:3 ratio is labeled as Sulfites. Nicked DNA is represented by II.

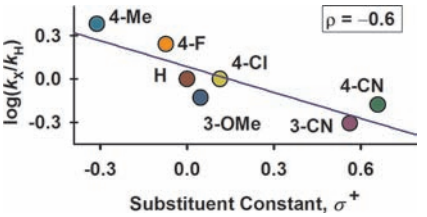

Figure 5. Hammett plot of rate constants for decomposition of **3a**–**9a** in pH 7.4 buffer at 37 °C. Linear regression analysis yielded ρ as −0.6 (R^2 = 0.75).

Here, we have presented a strategy for controlled and efficient generation of SO_2 under physiological pH and temperature under nonenzymatic conditions. There is a real need to understand precisely the biomolecular mechanisms of interaction of SO_2, as increased industrialization has resulted in larger populations being exposed to this gas[1] and SO_2's unique vasodilatory[3] and antimicrobial[4] effects might be exploited in developing novel therapeutic agents. It is anticipated that **3a** and its analogues, several of which are stable powders that release SO_2 in pH 7.4 buffer at predictable rates via a well-understood mechanism, would facilitate discerning these biological mechanisms.

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Supporting Information Available. Compound characterization data, computational data, procedures for decomposition and sulfur dioxide analysis, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

(12) Although a Hammett plot for a similar retro-Diels–Alder was not available for comparison, a previous report of a Diels–Alder reaction of 1-aryl-1,3-butadienes with maleic anhydride at 35 °C found ρ as −0.621. DeWitt, E. J.; Lester, C. J.; Ropp, G. A. *J. Am. Chem. Soc.* **1956**, *78*, 2101.

The authors declare no competing financial interest.