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Synthesis and antimycobacterial activity of prodrugs of sulfur dioxide (SO_2)

Satish R. Malwal [a], Dharmarajan Sriram [b], Perumal Yogeeswari [b], Harinath Chakrapani [a,*]

[a] Department of Chemistry, Indian Institute of Science Education and Research, Pune 411 008, India
[b] Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, India

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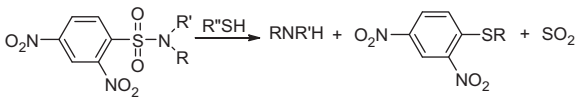
ABSTRACT

Here, we synthesized and studied a library of 2,4-dinitrophenylsulfonamides that closely resembled *N*-benzyl-2,4-dinitrophenylsulfonamide (**1**), a thiol-activated prodrug of sulfur dioxide (SO_2) which has shown high potency as a *Mycobacterium tuberculosis* (*Mtb*) inhibitory agent. The ability of these compounds to generate SO_2 in the presence of a thiol was evaluated. A good correlation between pK_{aH} of the corresponding amine and reactivity with thiols to generate SO_2 was found suggesting that the rate determining step of SO_2 generation involved protonation of the amine. Amongst analogues with measurable MICs, we also found a correlation between ability to generate SO_2 and *Mtb* growth inhibitory activity. Together, we report several thiol-mediated prodrugs of SO_2 which strongly inhibited *Mtb* growth (MIC <1 μg mL^{-1}) with potential for further development as tuberculosis drug candidates.

Tuberculosis causes millions of fatalities each year and in combination with HIV is proving to be a significant threat to global health.[1,2] Although several new promising drug candidates are under consideration for clinical use, the increasing incidences of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* (*Mtb*) necessitates new strategies for targeting this pathogen.[3–5] Recently, we reported that small amounts of sulfur dioxide (SO_2) inhibited *Mtb* growth.[6] SO_2 is an environmental pollutant that is toxic to humans at elevated levels;[7] chronic exposure to SO_2 induces oxidative stress and asthma-like symptoms.[8] SO_2 has diverse documented biological effects including damage to biomacromolecules such as proteins, lipids and DNA.[9–11] Oxidation of sulfur dioxide by metal ions produces sulfur trioxide radical, which possibly mediates damage to biomacromolecules.[11] Furthermore, sulfite, the anionic form of sulfur dioxide can break disulfide linkages to produce *S*-sulfonates.[10] Thus, SO_2 can participate in oxidative as well as reductive[12–14] processes under physiological conditions and may perturb redox equilibrium in cells. Recently, alteration of redox homeostasis in *Mtb* has been proposed as an effective mechanism for targeting this bacterium.[15] Thus, sulfur dioxide may have diverse mechanisms for cytotoxicity induction and multiple biological targets. Despite such well-documented deleterious effects, SO_2 (in the form of bisulfite, metabisulfite and sulfite) has also been routinely used as an antibiotic and antioxidant in the food industry[16–18] and is well tolerated in most individuals.[9,10] Thus, we proposed that SO_2 could be used as an anti-bacterial agent. In order to test this hypothesis, our strategy was to mask SO_2 as 2,4-dinitrobenzenesulfonamides,[19] which are candidates of thiol-activated SO_2 generation (Scheme 1).[6] We reported that amongst the compounds tested, the most potent *Mtb* inhibitor was found to be *N*-benzyl-2,4-dinitrophenylsulfonamide (**1**, Table 1) with a MIC of 0.05 μg/mL (0.15 μM), which was better than the MIC of clinically used isoniazid (0.05 μg/mL, 0.37 μM). This compound did not show significant toxicity against human embryonic renal cells at MIC (0.15 μM) against *Mtb*. Taken together, our results showed that SO_2 could be used as an anti-mycobacterial agent when masked as a suitable prodrug form.

Our preliminary results indicated that the rate of SO_2 generation affected inhibitory activity of this class of compounds. We found that small modifications to the structure of the amine resulted in a significant change in the half-lives of SO_2 generation ($t_{1/2}$ = 2–63 min). Thus, varying the amine structure could potentially help modulate anti-mycobacterial activity. Here, we proposed to synthesize and study a library of structural analogues of **1** in order to further understand the relationship between thiol-mediated sulfur dioxide generation and *Mtb* inhibitory activity. In order to study the effects of varying sterics and/or electronics on chemical and biological properties, analogues with comparable *c*log*P*s to **1** were synthesized.



Scheme 1. 2,4-Dinitrophenylsulfonamides are thiol-activated sources of sulfur dioxide (SO_2).

* Corresponding author. Tel.: +91 20 2590 8090; fax: +91 20 2589 9790.
E-mail address: harinath@iiserpune.ac.in (H. Chakrapani).

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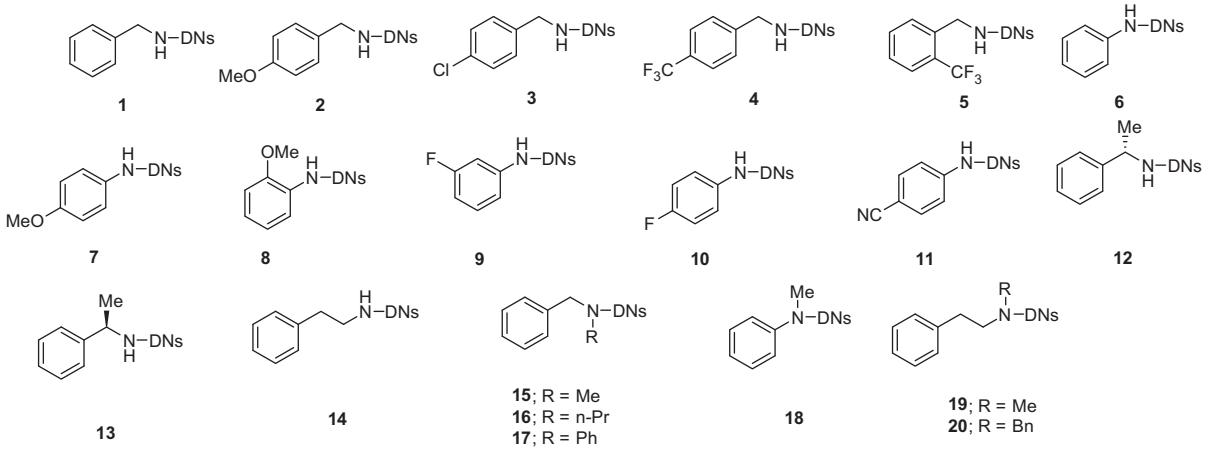
We prepared the benzylamine analogues **2**–**5** (Table 1, entries 2–5) with electron donating and electron withdrawing groups using a previously reported method of reaction of the corresponding amine with 2,4-dinitrophenylsulfonyl chloride (DNsCl). Similarly, aniline derivatives **6**–**11** were also prepared using the aforementioned procedure with substituents capable of perturbing the electronics of the amine (Table 1, entries 6–11). In order to study the effect of changing sterics around the nitrogen bearing the DNs group, we prepared **12** and **13** with an α-methyl substituent (Table 1, entries 12–13). The 1-phenyl-2-aminoethyl derivative **14** was prepared by treatment of the corresponding amine with DNsCl (Table 1, entry 14). Finally, the alkylated derivatives **15**–**20** were prepared by treatment of the corresponding alkyl halide with a suitable 2,4-dinitrosulfonamide (Table 1, entries 15–20).

Next, we evaluated cysteine-mediated SO_2 yields from this library of compounds (Table 1) by recording the yield of SO_2 30 min after commencement of the reaction with cysteine (10 equiv). SO_2 was determined as sulfite in pH 7.4 using an ion chromatograph equipped with a conductivity-based detector.[20] Under these reaction conditions, the benzylamine derivative **1** produced 100% SO_2 in 30 min (Table 1, entry 1). Presence of an electron withdrawing or electron donating group did not significantly affect the yield of SO_2 in comparison with **1** (Table 1, entries 2–5). The sulfanilide **6** produced 55 μM (55% yield) SO_2 under comparable reaction conditions (Table 1, entry 6). Amongst the aniline derivatives, we found a strong electronic effect on the yield of sulfur dioxide during decomposition of these derivatives. For example, the presence of an electron donating group enhanced the yield of SO_2 in comparison with aniline (Table 1, entries 7 and 8). Conversely, when an electron withdrawing group is present on the aromatic ring of aniline, the yield of SO_2 decreased in comparison with aniline (Table 1, entries 9–11). The effect was particularly strong in the presence of a 4-cyano group (**11**), whose sulfur dioxide yield (30 min) was 5% (Table 1, entry 11). In the presence of an additional

Table 1
Synthesis, calculated partition coefficients (*c*logP), thiol-mediated sulfur dioxide generation and anti-mycobacterial activities of 2,4-dinitrosulfonamides prepared in this study and related compounds



Entry	Compd	Mol. Wt.	Yield[a] (%)	*c*logP[b]	SO_2 yield, 30 min (μM)[c]	SO_2 yield, 5 min (μM)[c]	pK_{aH}[d]	MIC (μg mL^{-1})[e]	MIC (μM)
1	**1**	337.05	41	2.87	100	83	9.51	0.05[f]	0.15
2	**2**	367.33	33	2.78	84	74	9.50	0.25	0.68
3	**3**	371.75	54	3.58	96	77	9.44	0.4	1.07
4	**4**	405.31	43	3.75	75	79	9.45	0.4	0.98
5	**5**	405.31	54	3.75	84	81	9.23	1.56	3.84
6	**6**	323.28	30	2.76	55	37	4.64	3.13[f]	9.69
7	**7**	353.30	66	2.69	80	39	5.11	1.56[f]	4.4
8	**8**	353.30	70	2.69	86	57	4.42	3.13	8.85
9	**9**	341.27	70	2.93	24	5	3.22	25[f]	73
10	**10**	341.27	48	2.92	55	18	3.80	6.25	18.31
11	**11**	348.29	47	2.24	5	6	1.63	>50	>100
12	**12**	351.33	47	3.17	97	80	9.73	1.56	4.44
13	**13**	351.33	38	3.17	94	78	9.73	1.56	4.44
14	**14**	351.33	79	3.19	100	76	9.79	0.4	1.13
15	**15**	351.33	86	2.41	100	79	9.70	0.4[f]	1.10
16	**16**	379.38	73	3.47	96	68	9.90	3.13[f]	8.25
17	**17**	413.40	47	3.87	94	44	3.92	1.56	4.44
18	**18**	337.31	81	2.10	94	89	5.64	0.78	2.31
19	**19**	365.56	95	2.74	84	79	10.13	0.78	2.13
20	**20**	441.46	80	4.50	75	37	9.88	3.13	7.09
21	Isoniazid	137.13	—	−0.66	—	—	—	0.05	0.37
22	Ethambutol	204.31	—	2.08	—	—	—	1.56	7.63
23	Pyrizinamide	123.11	—	−0.67	—	—	—	6.25	50.8

[a] Yield is for reaction of the amine with 2,4-dinitrophenylsulfonyl chloride or alkylation of 2,4-dinitrophenylsulfonamide.
[b] Calculated using Chembiodraw Ultra.
[c] Sulfur dioxide as sulfite was quantified using an ion chromatograph equipped with a conductivity detector: yields are 5 min and 30 min after treatment of compound (100 μM) with 10 equiv of cysteine in pH 7.4 phosphate buffer.
[d] Values are for the corresponding amine and were calculated using Marvinsketch 5.7.1.
[e] Minimum inhibitory concentration (MIC) is the minimum concentration of the compound required to inhibit 99% of bacterial growth and was found against *Mycobacterium tuberculosis* $H_{37}R_v$ strain.
[f] Previously reported in Ref. 6.

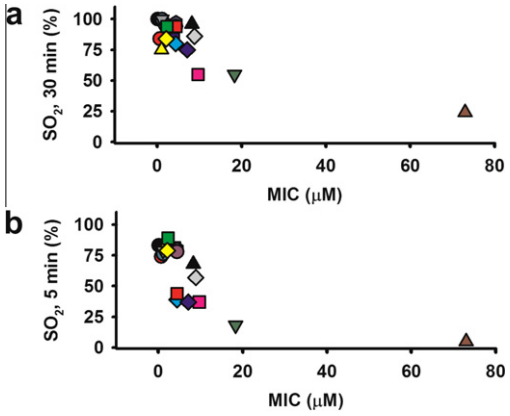


Figure 1. Relationship between sulfur dioxide yield generated during cysteine-mediated decomposition of 2,4-dinitrophenylsulfonamides prepared in this study and their *Mtb* inhibitory activity. (a) SO_2 yield was after 30 min; Spearman rank correlation analysis of SO_2 yield and MIC gave a Spearman correlation coefficient of −0.50 (*P*-value = 0.01). (b) SO_2 yield was after 5 min; Spearman rank correlation analysis of SO_2 yield and MIC gave $\rho = -0.69$ (*P*-value = 0.001).

alkyl, aryl, or methylene group, either comparable or slightly diminished yield of SO_2 in comparison with **1** was observed (Table 1, entries 12–20).

The ability of compounds in this library to inhibit *Mycobacterium tuberculosis* ($H_{37}R_v$) growth was evaluated using a reported protocol.[6] We found minimum inhibitory concentrations (MICs) ranged from 0.05 to >100 μg/mL (Table 1). While **1** was still the best *Mtb* inhibitor in this series, several derivatives **2–4**, **14**, **15**, **18** and **19** had potent *Mtb* inhibitory activities with MICs <1 μg/mL (Table 1, entries 2–4, 14–15, 18 and 19); these MICs were better than those of ethambutol and pyrizinamide, both clinically used tuberculosis drugs, evaluated under similar assay conditions (Table 1, entries 22 and 23).

Data analysis revealed that the most potent *Mtb* inhibitors generated elevated levels of SO_2 in 30 min (⩾75% yield). Spearman rank correlation analysis of MICs with sulfur dioxide yields (Fig. 1a) revealed a moderate negative correlation value of Spearman rank correlation coefficient $\rho = -0.50$ (*P*-value = 0.02) between MICs and SO_2 yields. Similar data collected for SO_2 yields after 5 min (Table 1), however, correlated well with MICs (Fig. 1b) $\rho = -0.69$ (*P*-value = 0.001). These results indicate a role for efficiency of SO_2 generation, which in turn is related to reactivity of the compound with thiols, in the observed *Mtb* inhibitory activity of this series of compounds.

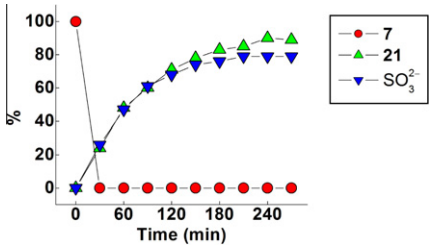


Figure 2. Kinetics of decomposition of **7** to produce sulfur dioxide and **21**. Disappearance of **7** and appearance of **21** was followed by HPLC analysis. Production of sulfite was monitored by ion chromatography analysis. Curve fitting afforded first order rate constant for: appearance of **21** as 0.011 min^{-1}; and appearance of sulfite as 0.014 min^{-1}.

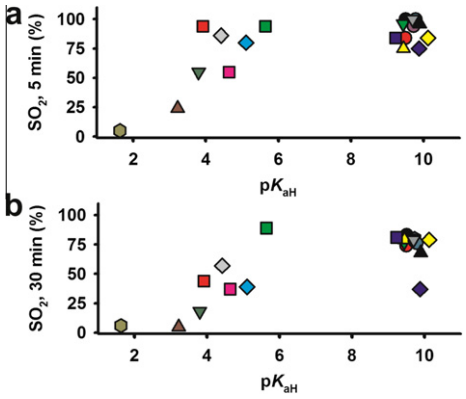
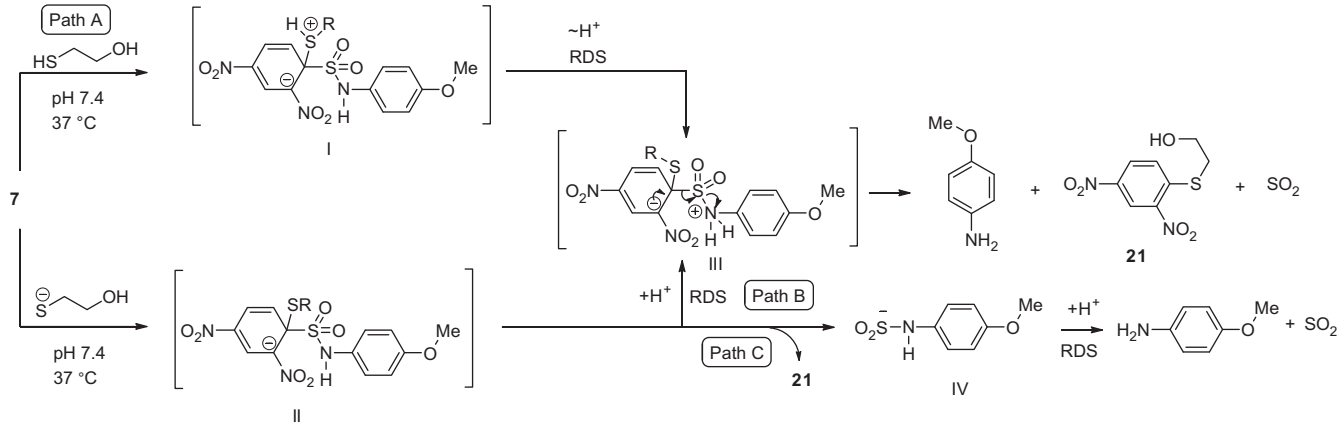


Figure 3. Relationship between sulfur dioxide generated during cysteine-mediated decomposition of 2,4-dinitrophenylsulfonamides prepared in this study and pK_{aH} of the amine from which the corresponding sulfonamide was prepared. (a) SO_2 yield was after 5 min; Pearson correlation analysis of SO_2 yield and pK_{aH} gave a correlation coefficient $r = -0.79$ (*P*-value <0.001) (b) SO_2 yield was after 30 min; Pearson correlation analysis of SO_2 yield and pK_{aH} gave a correlation coefficient $r = -0.71$ (*P*-value <0.0001).

Next, we performed a detailed kinetic analysis of thiol-mediated decomposition and sulfite generation from **7**. This compound was chosen as the rate of decomposition was amenable to simultaneous analysis of product decomposition and sulfur dioxide generation. Several possible mechanisms for the decomposition of this compound were considered (Scheme 2): path A, wherein thiol attack produces a Jackson–Meisenheimer intermediate I, which then rearranges via a transfer of proton to produce intermediate III, which then decomposes to simultaneously produces sulfur dioxide,



Scheme 2. Proposed mechanisms of decomposition of **7** to produce SO_2.

4-methoxyaniline and 2-hydroxyethylthio-2,4-dinitrobenzene (**21**).

Path B is an alternative wherein the attack on the aryl ring is by a thiolate to produce intermediate II, which then produces intermediate III by a protonation (Scheme 2). pK_as of thiols are typically in the range of 9–10 and hence would be expected to be in the form of thiolate ions in pH 7.4. However, protonation of II is still necessary as decomposition of II would produce an amide (ArN^-) anion which may not have physiological relevance. Finally, path C was considered wherein loss of **21** from III produces intermediate IV, which then produces SO_2 and 4-methoxyaniline by a slow decomposition step that involves protonation. In order to understand which pathway was dominant, **7** was treated with 2-mercaptoethanol (10 equiv) in pH 7.4 at 37 °C. HPLC analysis of the reaction mixture showed complete disappearance of **7** within 30 min of commencement of reaction (Fig. 2). We monitored the generation of **21** during the reaction course and we found a first order appearance of this compound during 6 h (Fig. 2).

Sulfite generation during this time period closely followed the generation of **21** suggesting that SO_2 and **21** were produced in the same decomposition step (Fig. 2). This observation suggests that path C was disfavored. As **7** decomposed much faster than appearance of SO_2, path A or B would be preferred with intermediate III generation (either through proton transfer from I or addition of proton to II) as the rate determining process (RDS). We found a good positive correlation $r = 0.79$ (P-value <0.0001) between pK_{aH} of the amine and SO_2 yields (5 min) and $r = 0.71$ (P-value <0.001) for SO_2 yields (30 min) during cysteine-mediated decomposition of 2,4-dinitrosulfonamides supporting that the proposed mechanism of formation of intermediate III as the rate determining step could be a general mechanism of thiol-mediated SO_2 generation from 2,4-dinitrophenylsulfonamides (Fig. 3).[21]

Nitro group reduction plays a major role in the action of PA-824, a nitroimidazole,[22–26] dinitrobenzamides (DNBs), and benzothiazinones (BTZs).[27–34] Although all these compounds have one or more nitro groups, their mechanisms of action and molecular targets in *Mtb* differ suggesting that reduction of an aromatic nitro group may have wide-ranging consequences to *Mtb* growth. At this time, involvement of nitro group reduction as a potential mechanism of action of 2,4-dinitrophenylsulfonamides reported in this work is unclear. However, as a majority of the potent *Mtb* inhibitors in our study were nearly completely decomposed in 30 min, it is perhaps less likely that they would stay unreacted, especially in the presence of (estimated) millimolar concentration of mycothiols in *Mtb*.[35] Thus, we report that anti-mycobacterial activity of closely related structural analogues of **1** with comparable *c*log Ps correlated well with the analogue's ability to generate sulfur dioxide upon treatment with cysteine. Future work will focus on identification of molecular targets and mechanisms of action of 2,4-dinitrophenylsulfonamides including **1**.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.048.

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