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Effect of alkyl group on transnitrosation of *N*-nitrosothiazolidine thiocarboxamides

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ABSTRACT

S-Nitrosoglutathione (GSNO) relaxes vascular smooth muscles, prevents platelet aggregation, and acts as a potential in vivo nitric oxide donor. 3-Nitroso-1,3-thiazolidine-4-thiocarboxamide (**1**), a *N*-nitrosothioproline analogue, exhibited a high GSNO formation activity. In this study, two compounds (**2** and **3**) based on compound **1** were newly synthesized by introducing either one or two methyl groups onto a nitrogen atom on the thioamide substituent in **1**. The pseudo-first-order rate constants (k_{obs}) for the GSNO formation for the reaction between the compound and glutathione followed the order **1** > **2**≒**3**. Thus, the introduction of a methyl group(s) onto the thioamide group led to a decrease in the transnitrosation activity. On the basis of density functional theoretical calculations, the transnitrosation for the *N*-nitrosothiazolidine thiocarboxamides was proposed to proceed via a bridged intermediate pathway. Specifically, the protonated compound **1** forms a bridged structure between the nitrogen atom in the nitroso group and two sulfur atoms—one in the ring and the other in the substituent. The bridged intermediate gives rise to a second intermediate in which the nitroso group is bonded to the sulfur atom in the thioamide group. Finally, the nitroso group is transferred to GSH to form GSNO.

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1. Introduction

Nitric oxide (NO) is important for many biological events, specifically in signalling pathways, immune responses, and vasodilation.[1] *S*-Nitrosothiols (RSNOs), such as *S*-nitrosoglutathione (GSNO), *S*-nitrosocysteine, and *S*-nitrosoalbumin, represent a circulating endogenous reservoir of NO and are potential donors of NO.[2–4] RSNOs have anti-platelet properties, a theoretical role in treating asthma, and potential for treating infectious diseases. Thus, RSNOs have been studied as NO donors in therapeutic drugs.[5–9]

GSNO is one of the most abundant *S*-nitrosothiols present in the body. It plays an important role in many important physiological functions of NO.[10] Specifically, GSNO has been tested as a therapeutic agent for clinical use.[5,11] However, GSNO has limited stability in aqueous solutions, and it readily degrades upon exposure to light.[10]

N-Nitrosamines have been reported to transfer a nitrosonium ion (NO^+) to other amines to form the corresponding *N*-nitroso derivatives.[12–15] Hence, as NO donors in the transnitrosation, *N*-nitrosamines can transnitrosate to a sulfur atom in proteins to form *S*-nitrosothiols. Ohwada et al. demonstrated that some bicyclic nitrosamines can generate GSNO without releasing NO.[16] We have previously focused on the GSNO formation via the transnitrosation of monocyclic *N*-nitrosamines. Although many *N*-nitrosodialkylamines are potent carcinogens, some *N*-nitrosamines, such as *N*-nitrosoproline and *N*-nitrosothioproline, are known to be non-mutagenic and non-carcinogenic.[17,18]

We have recently reported that the non-carcinogenic *N*-nitrosothioproline analogues transfer a nitroso group to glutathione (GSH) to form GSNO under acidic conditions.[19] The reaction rate of GSNO formation significantly increases when a thioamide group instead of a carboxyl group is introduced onto

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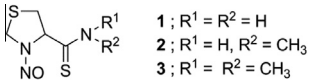


Figure 1. Structure of the alicyclic *N*-nitrosamines synthesized in this study.

the *N*-nitrosothioproline. *N*-Nitrosothioproline thiocarboxamide is a 3-nitroso-1,3-thiazolidine-4-thiocarboxamide (**1**) with a sulfur atom in its substituent and in its ring. To increase the rate of GSNO formation, a series of *N*-nitrosothiazolidine thiocarboxamides (**1**–**3**) were synthesized (Fig. 1). The transnitrosation mechanism for *N*-nitrosothiazolidine thiocarboxamide **1** was proposed on the basis of the computational calculations.

2. Results

2.1. Chemistry

To increase the nucleophilicity of the sulfur atom in the thioamide group in **1**, one or two electron-donating methyl groups were introduced to the nitrogen atom on the thioamide group. We designed a *N*-methylthioamide-substituted compound (**2**) and a *N*,*N*-dimethyl-thioamide-substituted compound (**3**).

The procedures for synthesizing the compounds (**2** and **3**) are shown in Scheme 1. Methyl 1,3-thiazolidine-4-carboxylate was prepared via a reaction between thioproline and thionyl chloride in methanol. The methyl 1,3-thiazolidine-4-carboxylate solid was directly added to the methylamine-containing aqueous solution to obtain *N*-methyl-1,3-thiazolidine-4-carboxamide. The preparation of *N*,*N*-dimethyl-1,3-thiazolidine-4-carboxamide was performed in the same manner using dimethylamine. The nitrogen atom in the ring was protected with a *tert*-butoxycarboxyl (Boc) group. Under ultrasonication, carboxamides in the Boc compounds were directly thiolated with diphosphorous pentasulfide to provide the thioamides.[20] The Boc group was then removed under acidic conditions and subsequently nitrosated with sodium nitrite to afford the desired compound.

Compound **1** was synthesized according to the method described in the literature[19] by thiolating 1,3-thiazolidine-4-carboxamide using ultrasonication. However, the product yield of 1,3-thiazolidine-4-thio-carboxamide was very low (26%). Then, **1** was prepared after the Boc group was introduced using the same method used for **2** and **3** (Scheme 1).

2.2. Transnitrosation activity evaluation based on GSNO formation

The transnitrosation activity of the compounds was evaluated in terms of their capacity to form GSNO. The pseudo-first-order rate constants (k_{obs}) were determined using the slope of the linear

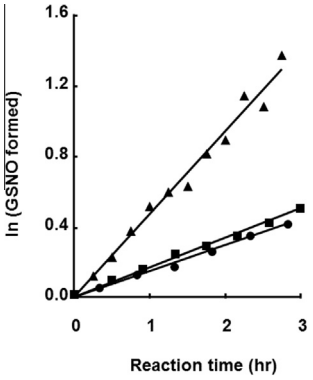


Figure 2. Formation of GSNO from the reaction of *N*-nitrosamines (**1**–**3**) with GSH. The reaction conditions were as follows: *N*-nitrosamine **1** (▲), **2** (■), or **3** (●), 0.45 mM; GSH, 5.0 mM; pH 1.5; 37 °C.

Table 1
Reaction rate constant (k_{obs}) and GSNO yield in transnitrosation

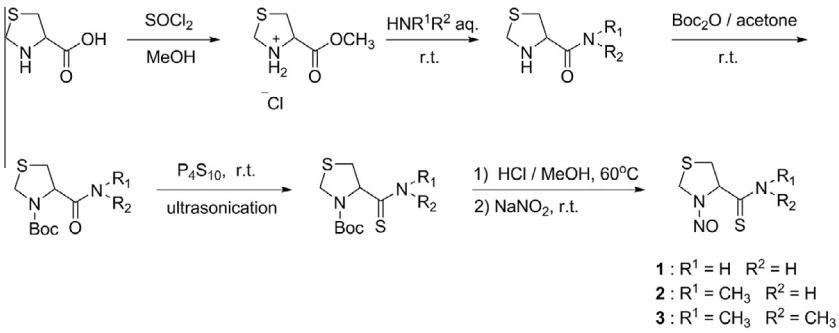
N-Nitrosamine	k_{obs}[b] ($\times 10^{-7}$/s)	GSNO[c] (mM)	*N*-Nitrosamine consumption[c] (mM)	GSNO yield[c] (%)
1[a]	1301	0.43	0.45	95
2[a]	464	0.38	0.45	85
3[a]	405	0.38	0.45	84

[a] The reaction conditions are as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.5; 37 °C; in the dark.
[b] The k_{obs} for GSNO formation was calculated from the slope in $\ln\{[GSNO_\infty]/([GSNO_\infty] - [GSNO])\}$ versus time, where GSNO and $GSNO_\infty$ refer to the GSNO concentration at the time and the final concentration (0.45 mM), respectively. The k_{obs} were determined by the method of the least-squares.
[c] Data were acquired after 24 h.

relationship between the GSNO concentration and the reaction time (Fig. 2). The transnitrosation results are summarised in Table 1. The k_{obs} values followed the order **1** > **2**≒**3** (Table 1). The GSNO yields after 24 h of reaction time were calculated by dividing the [GSNO formation] by the [*N*-nitrosamine consumption] (Table 1). The GSNO yields were 95% for **1**, 85% for **2**, and 84% for **3**. These results indicate that the transnitrosation to GSH proceeded selectively without decomposition of the compound.

2.3. The pH-rate profile for GSNO formation

The logarithms of k_{obs} for GSNO formation in the reaction of **1** with GSH were linearly correlated with the oxonium-ion concentration. The pH-rate ($\log k_{obs}$) profile gave a slope of -1.32 ± 0.43 (Fig. 3). Thus, the transnitrosation proceeded via a specific acid catalysis and the protonated **1** was involved in the rate-determining step.[21]



Scheme 1. Synthesis of *N*-nitrosothiazolidine thiocarboxamides.

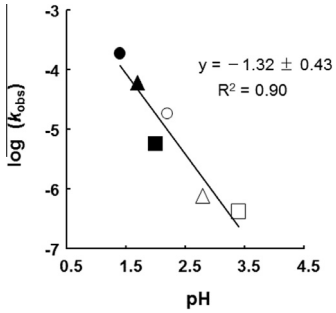


Figure 3. The pH-rate profile for the formation of GSNO (k_{obs}) in **1** and GSH. The reaction conditions were as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.4 (●), 1.7 (▲), 2.0 (■), 2.2 (○), 2.8 (△), or 3.3 (□); 37 °C.

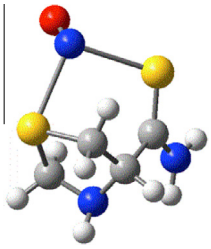


Figure 4. DFT-optimised structure of the protonated **1b** (B3LYP/6-311G(d) level).

2.4. Theoretical calculations of *N*-nitrosothiazolidine thiocarboxamides

Density functional theoretical (DFT) calculations were performed to find out the thermodynamically most favored pathway of the transnitrosation mechanism and to gain insight into some key intermediate or transition states involved in the reaction. The geometries of the species (**1**) in the transnitrosation reaction were optimised in the computational calculations using the double hybrid density functional B3LYP and the basis set 6-311G(d), as implemented in Gaussian09. The single-point energy was subsequently calculated.[22] The free-energy difference (ΔG) was obtained by subtracting the single-point energy of a former form from that of following intermediate in the stepwise reaction.

In previous studies, *N*-nitrosoproline acts as an NO donor in the presence of thiourea, whereas **1** containing sulfur atoms both in the ring and as a substituent exhibited high activity for GSNO formation without thiourea.[19] The data indicate that the intramolecular sulfur atoms in the *N*-nitrosamines were involved in the transnitrosation, and we then proposed a presumable transnitrosation pathway for *N*-nitrosamine **1** (Scheme 2).

Protonation of nitrogen atom attaching the nitroso group and protonation of oxygen atom are present at an equilibrium process. The initial protonation site is the nitrogen in the *N*-nitrosothiazolidine ring which was determined by the comparison of the energies of protonated isomers calculated by the DFT. The energy of the protonation at the *N*-nitrosothiazolidine ring is more stable than that of protonation at the oxygen atom of NO group (+9.6 kcal mol^{-1}) and amine moiety of thiocarboxamide group (+19.3 kcal mol^{-1}). The nitroso group in *N*-protonated **1** is then transferred to a sulfur atom of one of the thioamide groups or to the sulfur atom of the ring. The two routes are presented for paths A and B, which lead to **c** and **d**, respectively. Then, the intermediate transfers the nitroso group to GSH to form GSNO.

Interestingly, the optimised structure of the protonated **1** (**1b**) was a bridged form between the nitrogen atom in the nitroso group and two sulfur atoms—one in the ring and the other on the substituent (Fig. 4).

A transition state (TS) is expected to be similar to the bridged intermediate. The transition state is proposed to include a nitroso group bonded with three intramolecular atoms (two sulfur atoms and the nitrogen atom that attaches to the nitroso group). The TS was validated by an intrinsic reaction coordinate (IRC) calculation. The activation energy in gas phase has been determined and confirmed by the IRC calculations. The energy diagrams including TS energies for the main reaction course are given in Figure 5.

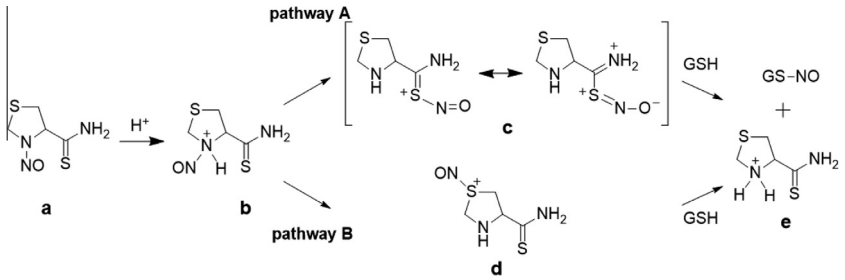
The stability of the intermediates **c** and **d** is compared, and the energies of the intermediate **c** is lower than that of **d** (Fig. 5). The data indicates that the path A for **1** is preferred.

3. Discussion

Because GSNO is a potential therapeutic agent,[5,10] we focused on the GSNO formation from *N*-nitrosamines by transnitrosation. Non-mutagenic *N*-nitrosothioproline was used as the basic structure for developing new transnitrosating *N*-nitrosamines.[19] Compounds **2** and **3**, which were introduced with one or two methyl groups, respectively, on the thioamide nitrogen in **1**, were newly synthesized, and *N*-nitrosothiazolidine thiocarboxamides could transnitrosate to GSH to form GSNO. Contrary to the expectation that the introduction of the electron-donating methyl group(s) into the thioamide increases their nucleophilicity and is followed by an increase in the k_{obs} for the GSNO formation, compounds **2** and **3** decreased the k_{obs} for the GSNO formation.

To elucidate the transnitrosation mechanism for **1**, the effect of pH on k_{obs} was investigated and a computational study was performed. The pH-rate profile with a slope of −1.32 ± 0.43 indicated that protonated form of the *N*-nitrosamine was appeared in the rate-determining step.[21] The pH-dependency of GSNO formation was consistent with the formation step of the bridged **1b** in TS as shown in Figure 5.

In the computational studies of the protonated **1**, the optimised structure was a bridged form between the nitrogen atom in a



Scheme 2. Proposed intermediates in the transnitrosation mechanism for *N*-nitrosothiazolidine thiocarboxamide **1** under acidic conditions.

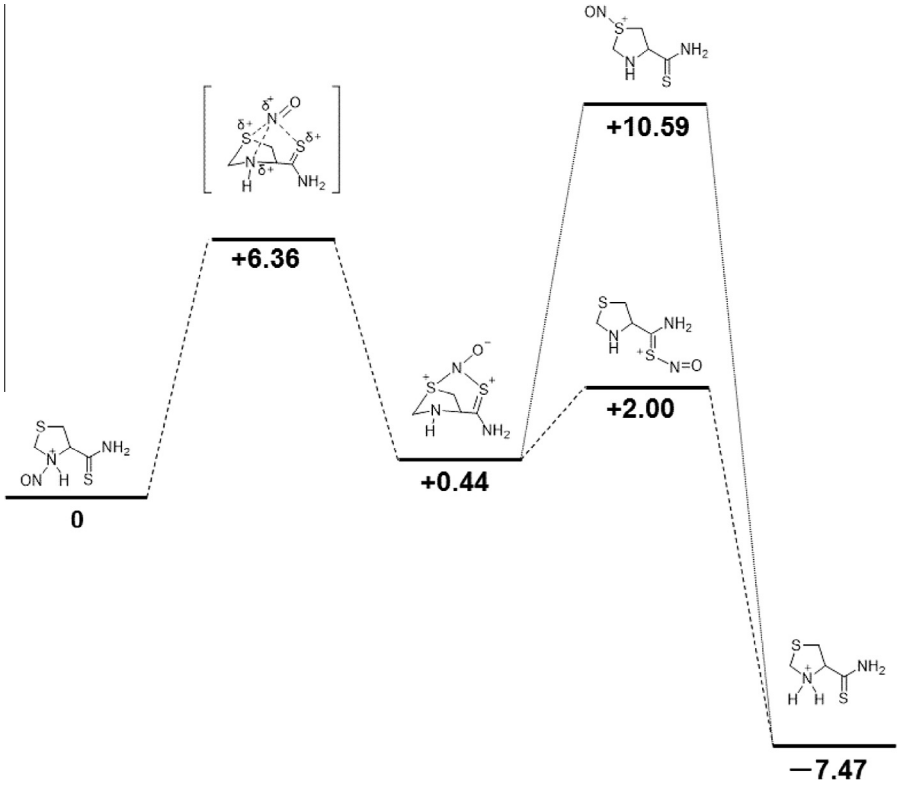
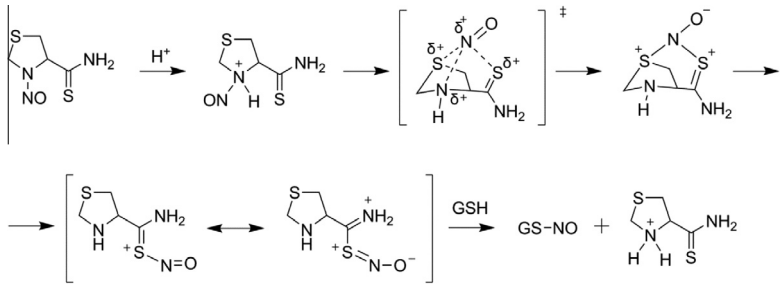


Figure 5. Potential energies of transnitrosation between *N*-nitrosamine **1** and GSH at the B3LYP/6-311(g) level.



Scheme 3. Transnitrosation mechanism for *N*-nitrosothiazolidine thiocarboxamide **1** under acidic conditions.

nitroso group and two sulfur atoms, one in the ring and the other in the substituent (Fig. 4). The formation of the bridged structure is strongly supported, because the GSNO formation of the *N*-nitrosothiazolidine thiocarboxamides is significantly enhanced by introducing two sulfur atoms into the molecule. A few reports indicates that the S—N(=O)—S structure is formed by transnitrosation with *S*-nitrosothiol and another thiol.[23–25] The reported data were similar to our data in that an intramolecular S—N(=O)—S bridged intermediate was formed. Although the bridged structure is highly distorted, it is a bicyclic structure with a five-membered ring and a six-membered ring.

According to the computations, the intermediate **c** for path A or **d** for path B was assumed to exist, and path A was preferred (Fig. 5). The computational data was in good agreement with resonance stability of **C** in path A.

Unexpectedly, the k_{obs} for GSNO formation of **2** and **3** were lower than that of **1**. Introduction of methyl group into thioamide group caused decrease the GSNO formation. Generally, the introduction of a methyl group onto a thioamide group enhances the partial N=N double-bond character via the electron-donating ability of the methyl group, increases the stability of intermediates, and finally raises the GSNO formation. We considered a reason to decrease the k_{obs} in **2** and **3**, the bridged form and/or the S (thioamide)—NO form was unstable in **2** and **3**. The instability in **2** and **3** structures is due to the steric hindrance between the ring and the methyl group. Thus, we assumed that the electronic effect of the methyl group in their intermediates increased the stability, whereas the steric hindrance between the methyl group and the ring led a decrease the stability of their intermediates of **2** and **3**.

N-Nitrosamine **1** has the highest GSNO yield (95%) even though the intermediate **1b** was stable. Therefore, we thought that a rate-determining step for **2** and **3** was different from that for **1** and a formation step of S(thioamide)—NO structure was the rate-determining step. In the reaction mixture, compounds **2** and **3** could exist in their bridged forms, resulting in lower GSNO yields (85% for **2** and 84% for **3**).

Our experimental and theoretical evidence clearly confirmed the mechanism in Scheme 3 for the transnitrosation of the *N*-nitrosamine **1**.

In summary, the transnitrosation step was characterised. It begins with the protonation of the *N*-nitrosamine and is followed by the formation of a stable intermediate. The transition state is

similar to the bridged form. The transition state is confirmed to include a nitroso group bonded with three intramolecular atoms (two sulfur atoms and the nitrogen atom that attaches to the nitroso group). The bridged intermediate then gives rise to a second intermediate in which the nitroso group is bonded to a sulfur atom in the thioamide group. Finally, the nitroso group is transferred to GSH to form GSNO.

4. Conclusion

GSNO is a potential therapeutic agent; however, its poor stability is problematic. Therefore, we focused on a series of *N*-nitrosothiazolidine thiocarboxamides for GSNO formation. Three newly synthesized *N*-nitrosothiazolidine thiocarboxamides (**1**–**3**) exhibited transnitrosation activity under acidic conditions. The activity of the GSNO formation for **1** was higher than that for **2** and **3**. In the transnitrosation mechanism, a protonated structure is formed as a transition state. An intramolecular S—N(=O)—S bridge structure was characterised as an intermediate based on the result of DFT calculations. Thus, we propose that the transnitrosation activity of *N*-nitrosothiazolidine thiocarboxamides can enhance by introduction of a sulfur-containing substituent, which has an electron-donating effect and less steric hindrance. The studied compounds are therapeutic candidates for GSNO-related diseases.

5. Experimental

5.1. Chemicals

L-Thioproline was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Diphosphorous pentasulfide was purchased from Sigma–Aldrich Co., Inc. (St. Louis, MO, USA). Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA) was acquired from Dojindo Laboratories (Kumamoto, Japan). Unless otherwise noted, chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) at the highest available purity and were used without further purification.

5.2. General procedure

The reaction progress was monitored using thin-layer chromatography (TLC) on silica gel 60 F_{254} (0.25 mm, Merck) and aluminium oxide 150 F_{254} neutral plates (Merck). Column chromatography was performed using silica gel 60 (0.01–0.063 mm, Merck) or aluminium oxide 90 (0.063–0.200 mm, Merck) active neutral solid phases. Melting points were determined using a Yanaco micro-melting-point apparatus without correction. HPLC was performed using a Shimadzu LC system [SPD-20A UV spectrometric detector, Shiseido Capcell Pak column (5 μm, 250 × 4.6 mm)]. The NMR spectra were recorded with a JEOL JNM-LA400 spectrometer (Tokyo, Japan). The chemical shifts are expressed in ppm and were shifted downfield from TMS. The high-resolution mass spectra were collected using a JEOL JMS-SX102A mass spectrometer. GSNO was synthesised using the Hart method [λ_{max} (H_2O): 335 nm (ε = 918) (lit. λ_{max}: 336 nm (ε = 922)].[26]

5.3. General procedure for alicyclic *N*-nitrosamines

5.3.1. Preparation of *N*-alkyl-1,3-thiazolidine-4-carboxamide by alkylamines and 1,3-thiazolidine-4-carboxamide

Methyl 1,3-thiazolidine-4-carboxylate hydrochloride and 1,3-thiazolidine-4-carboxamide were prepared according to a previously reported procedure.[19] An excess of the corresponding *N*-alkylamine aqueous solution was added dropwise to methyl 1,3-thiazolidine-4-carboxylate hydrochloride in an ice bath and stirred at room temperature until the starting material disappeared in the TLC chromatograms. The reaction mixture was extracted with $CHCl_3$, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified on a silica-gel column to afford the desired product as a single compound.

N-Methyl-1,3-thiazolidine-4-carboxamide; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.51 (d, J = 1.2, 4.1 Hz, 1H, S-CH_2-N), 4.07 (dq, J = 1.5, 11.2 Hz, 1H, CONHCH$_3$), 4.02 (d, J = 5.6 Hz, 1H, S-CH_2-N), 3.96 (dd, J = 1.7, 6.6 Hz, 1H, N-C*H*-CO), 3.31 (dd, J = 1.7, 12.0 Hz, 1H, CH-CH_2-S), 3.19 (ddd, J = 1.7, 1.9, 6.6 Hz), 2.85 (d, J = 1.2 Hz, 3H, NHCH_3).

N,*N*-Dimethyl-1,3-thiazolidine-4-carboxamide; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (d, J = 9.5 Hz, 1H, S-CH_2-N), 4.11 (d, J = 9.5 Hz, 1H, S-CH_2-N), 3.89 (dd, J = 6.6, 9.1 Hz, 1H, N-C*H*-CO), 3.20 (dd, J = 6.7, 10.0 Hz, 1H, CH-CH_2-S), 3.11 (s, 3H, N-CH_3), 3.01 (s, 3H, N-CH_3), 2.68 (t, J = 10.0 Hz, 1H, CH-CH_2-S).

5.3.2. Preparation of 3-*tert*-butoxycarbonyl-1,3-thiazolidine-4-carboxamides

A solution of di-*tert*-butyl dicarbonate in acetone was added to a solution of the corresponding 1,3-thiazolidine-4-carboxamides in acetone under nitrogen atmosphere. The reaction mixture was stirred at room temperature until the starting material disappeared in the TLC chromatograms. After the reaction mixture was concentrated under reduced pressure, the crude product was purified on a silica-gel column to afford the single desired product.

3-*tert*-Butoxycarbonyl-1,3-thiazolidine-4-carboxamide; ^{1}H NMR (400 MHz, methanol-d_4) δ 5.45 (br, 1H, N-C*H*-CO), 4.63 (br, 1H, S-CH_2-N), 4.44 (br, 1H, S-CH_2-N), 3.48 (br, 1H, C-CH_2-S), 3.13 (br, 1H, C-CH_2-S), 1.46 (s, 9H, *tert*-butyl).

3-*tert*-Butoxycarbonyl-*N*-methyl-1,3-thiazolidine-4-carboxamide; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.65 (d, *br*, J = 9.5 Hz, 2H, N-C*H*-CO, S-CH_2-N), 4.35 (br, 1H, S-CH_2-N), 3.42 (br, 1H, C-CH_2-S), 3.20 (br, 1H, C-CH_2-S), 2.85 (d, J = 4.6 Hz, 3H, N-CH_3), 1.48 (s, 9H, *tert*-butyl).

3-*tert*-Butoxycarbonyl-*N*,*N*-dimethyl-1,3-thiazolidine-4-carboxamide; ^{1}H NMR (400 MHz, methanol-d_4) δ 5.15 (br, 1H, N-C*H*-CO), 4.89 (br, 1H, S-CH_2-N), 4.75 (br, 1H, S-CH_2-N), 4.52 (d, J = 8.8 Hz, 1H, C-CH_2-S), 3.31 (dd, J = 3.7, 11.2 Hz, 1H, C-CH_2-S), 3.12 (s, 3H, N-CH_3), 2.99 (s, 3H, N-CH_3), 1.51 (s, 9H, *tert*-butyl).

5.3.3. Preparation of 3-*tert*-butoxycarbonyl-1,3-thiazolidine-4-thiocarboxamides

Diphosphorous pentasulfide (1 equiv) was added to a solution of the corresponding 3-*tert*-butoxycarbonyl-1,3-thiazolidine-4-carboxamide in THF.[20] The reaction mixture was ultrasonicated below 25 °C until the starting material disappeared in the TLC chromatograms. After the addition of acetone, the reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified on silica-gel columns to afford the single desired product.

3-*tert*-Butoxycarbonyl-1,3-thiazolidine-4-thiocarboxamide; white needle crystals (chloroform); yield 47%; mp 174.0–176.0 °C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (br, 1H, CSNH_2), 5.03 (t, J = 5.5 Hz, 1H, N-C*H*-CS), 4.68 (d, J = 9.5 Hz, 1H, S-CH_2-N), 4.48 (br, 1H, S-CH_2-N), 3.45 (br, 2H, C-CH_2-S), 1.48 (s, 9H, *tert*-butyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.41 (*C*=S), 154.01 (*C*=O), 82.36 (*C*(CH$_3$)$_3$), 69.41 (N-*C*H-CS), 50.32 (S-CH$_2$-N), 37.94 (C-CH$_2$-S), 28.20 (C(*C*H$_3$)$_3$); HRMS (EI) 248.0650 (calcd for $C_9H_{16}N_2O_2S_2$ 248.0653).

3-*tert*-Butoxycarbonyl-*N*-methyl-1,3-thiazolidine-4-thiocarboxamide; white needle crystals (chloroform and hexane); yield 81%; mp 149.0–152.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (br, 1H, N*H*), 5.07 (t, J = 5.3 Hz, 1H, N-C*H*-CS), 4.65 (d, J = 9.8 Hz, 1H, S-CH_2-N), 4.45 (d, J = 8.8 Hz, 1H, S-CH_2-N), 3.44 (br, 2H, C-CH_2-S),

3.22 (d, J = 4.9 Hz, 3H, N-CH_3), 1.46 (s, 9H, *tert*-butyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.59 (C=S), 154.09 (C=O), 82.22 ($C(CH_3)_3$), 70.16 (N-CH-CS), 50.46 (S-CH_2-N), 37.73 (C-CH_2-S), 32.66 (NCH_3), 28.20 ($C(CH_3)_3$); HRMS (EI) 262.0813 (calcd for $C_{10}H_{18}N_2O_2S_2$ 262.0810).

3-*tert*-Butoxycarbonyl-*N*,*N*-dimethyl-1,3-thiazolidine-4-thiocarboxamide; white needle crystals (chloroform and hexane); yield 49%; mp 56.5–58.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (t, J = 6.8 Hz, 1H, N-CH-CS), 5.13 (br, 1H, N-CH-CS), 4.85 (br, 1H, S-CH_2-N), 4.74 (br, 2H, S-CH_2-N), 3.51 (s, 6H, N-CH_3), 3.45 (s, 3H, N-CH_3), 3.37 (d, J = 7.6 Hz, 1H, C-CH_2-S), 3.35 (d, J = 7.6 Hz, 1H, C-CH_2-S), 3.23 (br, 2H, C-CH_2-S), 1.47 (s, 9H, *tert*-butyl), 1.40 (s, 9H, *tert*-butyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.64, 202.67 (C=S), 153.17, 152.41 (C=O), 80.95 ($C(CH_3)_3$), 64.00, 50.59 (N-CH-CS), 50.20, 45.21 (S-CH_2-N), 41.62, 41.39 (NCH_3), 37.34, 36.18 (C-CH_2-S), 28.36 ($C(CH_3)_3$); HRMS (EI) 276.0964 (calcd for $C_{11}H_{20}N_2O_2S_2$ 276.0966).

5.3.4. Preparation of 3-nitroso-1,3-thiazolidine-4-thiocarboxamides (1, 2, 3)

The corresponding 3-*tert*-butoxycarbonyl-1,3-thiazolidine-4-thiocarboxamide was dissolved in methanol and was acidified to pH 3 by the addition of 1 M HCl. The reaction mixture was stirred at 60 °C until the starting material disappeared in the TLC chromatograms. After the solution cooled to room temperature, sodium nitrite (1 equiv) was added and the resulting mixture was stirred for 1 h until the deprotected compound disappeared. The reaction mixture was extracted with CH_2Cl_2, and the combined organic layers were washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified on silica-gel columns to afford the single desired compound.

1; white needle crystals (chloroform); yield 13%; mp 121.5–122.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (br, 1H, *E*-CSNH_2), 9.79 (br, 1H, *Z*-CSNH_2), 9.47 (br, 1H, *E*-CSNH_2), 9.39 (br, 1H, *Z*-CSNH_2), 5.88 (dd, J = 3.7, 7.3 Hz, 1H, *E*-N-CH-CS), 5.86 (d, J = 11.0 Hz, 1H, *Z*-S-CH_2-N), 5.26 (d, J = 10.3 Hz, 1H, *Z*-S-CH_2-N), 4.99 (t, J = 7.9 Hz, 1H, *Z*-N-CH-CS), 4.87 (d, J = 12.2 Hz, 1H, *E*-S-CH_2-N), 4.56 (d, J = 12.2 Hz, 1H, *E*-S-CH_2-N), 3.63 (dd, 1H, *E*-CH-CH_2-S), 3.58 (dd, J = 7.9, 12.2 Hz, 1H, *Z*-CH-CH_2-S), 3.33 (dd, 1H, *E*-CH-CH_2-S), 3.20 (dd, J = 7.9, 11.6 Hz, 1H, *Z*-CH-CH_2-S).

2; white needle crystals (chloroform and hexane); yield 7%; mp 149.0–149.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (dd, J = 1.7, 4.9 Hz, 1H, *E*-N-CH-CS), 5.75 (d, J = 10.0 Hz, 1H, *Z*-S-CH_2-N), 5.22 (t, J = 3.7 Hz, 1H, *Z*-N-CH-CS), 5.19 (s, 1H, *E*-S-CH_2-N), 4.52 (d, J = 12.2 Hz, 1H, *E*-S-CH_2-N), 4.02 (m, 1H, *E*-CH-CH_2-S), 4.00 (m, 1H, *Z*-CH-CH_2-S), 3.54 (dd, J = 6.8, 6.6 Hz, 1H, *E*-C-CH_2-S), 3.42 (dd, J = 7.8, 7.8 Hz, 1H, *Z*-C-CH_2-S), 3.25 (d, J = 4.9 Hz, 3H, *E*-N-CH_3), 3.16 (d, J = 4.9 Hz, *Z*-N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4 (*E*-CSNH$_2$), 200.6 (*Z*-CSNH$_2$), 70.7 (*E*-N-CH-CS), 66.2 (*Z*-N-CH-CS), 53.5 (*Z*-S-CH_2-N), 47.8 (*E*-S-CH_2-N), 35.2 (*E*-S-CH$_2$-C), 34.8 (*Z*-S-CH$_2$-C); HRMS (EI) 191.0188 (calcd for $C_5H_9N_3OS_2$ 191.2745).

3; a tan-yellow crystals (chloroform and hexane); yield 62%; mp 97.0–97.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.03 (t, J = 7.1 Hz, 1H, *Z*-S-CH_2-N), 5.78 (d, J = 4.9 Hz, 1H, *E*-S-CH_2-N), 5.48 (d, J = 1.0 Hz, 1H, *E*-S-CH_2-N), 5.33 (t, J = 7.6 Hz, 1H, *E*-N-CH-CS), 5.18 (d, J = 11.7 Hz, 1H, *Z*-S-CH_2-N), 3.67 (dd, J = 6.1, 6.4 Hz, 1H, *Z*-CH-CH_2-S), 3.55 (s, 3H, N-CH_3), 3.54 (s, 3H, *Z*-N-CH_3), 3.53 (s, 3H, *Z*-N-CH_3), 3.38 (dd, J = 1.0, 7.3 Hz, 1H, *E*-CH-CH_2-S); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4 (*E*-CSNH$_2$), 200.6 (*Z*-CSNH$_2$), 70.7 (*E*-N-CH-CS), 66.2 (*Z*-N-CH-CS), 53.5 (*Z*-S-CH_2-N), 47.8 (*E*-S-CH_2-N), 35.2 (*E*-S-CH$_2$-C), 34.8 (*Z*-S-CH$_2$-C); HRMS (EI) 205.0345 (calcd for $C_6H_{11}N_3OS_2$ 205.0344).

5.4. Reaction of 1–3 with GSH

The GSH (50 mM) and DTPA (24 μM) were dissolved in 0.1 M sodium phosphate buffer (pH 7.4). The *N*-nitroso compound (4.5 mM) was dissolved in acetonitrile. Aliquots of GSH (300 μL, 5.0 mM) and DTPA (150 μL, 1.2 μM) were mixed, and the pH of the resulting mixture was adjusted to 1.5 with 0.1 M HCl for a total volume of 2.7 mL. The reaction was initiated by adding a solution of the compound (300 μL, 0.45 mM) at 37 °C. The aliquots were collected at specified intervals, and the GSNO yield was determined using HPLC with a Shiseido UG80 (5 μm, 150 × 4.6 mm^2) column with methanol/0.05% trifluoroacetic acid (5:95) as the eluent at 1.0 mL/min at 335 nm.

5.5. Kinetic analysis of the transnitrosation reactions

The reaction rate constants were determined by measuring the GSNO formation at 37 °C. The pseudo-first-order rates (k_{obs}) were determined using graphical analysis of the initial linear portion of the curve obtained from a plot of $\ln\{[GSNO_\infty]/([GSNO_\infty] - [GSNO])\}$ versus t. The theoretical value for $GSNO_\infty$ was the initial concentration of the *N*-nitroso compound. All rate values were calculated using the least-squares method, and each experiment was conducted in three separate trials.

The quantity of *N*-nitroso that remained was quantified simultaneously with the quantity of GSNO formed. The yield of S-nitrosation from *N*-nitrosamines after the 24 h reaction time was calculated by dividing the percentage of GSNO that was formed by the percentage of the *N*-nitroso compound.

5.6. pH-rate profile

The GSH and DTPA solutions were adjusted to the desired pH. The reaction mixtures were prepared and analysed as previously described.

5.7. Theoretical calculations

DFT calculations were performed using Gaussian 09.55. The calculations were performed on a 32-processor QuantumCube at the B3LYP/6-311+G(d) level of theory by applying the polarizable continuum model (PCM) using the integral equation formalism variant (IEF-PCM).[22]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.09.008.

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