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Redox Regulation of Protein Tyrosine Phosphatase 1B (PTP1B): A Biomimetic Study on the Unexpected Formation of a Sulfenyl Amide Intermediate

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Abstract: The effect of steric and electronic environments around the sulfur and nitrogen atoms and the role of nonbonded S...O/N interactions on the cyclization reactions of amide substituted benzene sulfenic acids are described. The reaction profiles and the role of different substituents on the cyclization are investigated in detail by theoretical calculations. It is shown that the synthetic thiols having *ortho*-amide substituents may serve as good models for the enforced proximity of the amide and cysteine thiol groups at the active site of protein tyrosine phosphatase 1B (PTP1B). However, some of the sulfenic acids derived from such models do not effectively mimic the cyclization of protein sulfenic acids. This is mainly due to the requirement of very high energy for breaking the S–O bond to form a planar five-membered ring of isothiazolidinone. It is shown that the sulfenic acid having two substituents—an amide moiety and a heterocyclic group—in the *ortho*-positions undergoes a rapid cyclization reaction to produce the corresponding sulfenyl amide species. These studies reveal that the introduction of a substituent at the 6-position of the benzene ring enhances the cyclization process not only by facilitating a closer approach of the –OH group and the backbone –NH moiety but also by increasing the electrophilicity of the sulfur atom in the sulfenic acid.

Introduction

Protein tyrosine phosphatases (PTPs) regulate signal transduction pathways involving tyrosine phosphorylation, and these enzymes have been implicated in the development of cancer, diabetes, rheumatoid arthritis, and hypertension.¹ In particular, the structure and function of the mammalian protein tyrosine phosphatase 1B (PTP1B) have attracted significant interest in recent years due to the importance of this enzyme in insulin signaling.² The reaction mechanism of PTP1B involves a nucleophilic attack of a catalytically active cysteine residue (Cys215) at a phosphotyrosine (pTyr) substrate, resulting in a covalent phosphocysteine intermediate that is subsequently hydrolyzed by a water molecule (Figure 1A).³

Recent studies suggest that the cellular redox state is involved in regulating tyrosine phosphatase activity through the reversible oxidation of the catalytic cysteine to the corresponding sulfenyl acid (Cys-SOH).⁴ The sulfenyl acid reacts with thiols such as

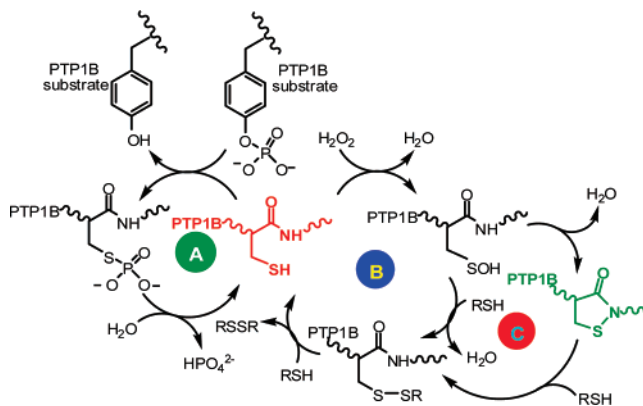


Figure 1. (A) Dephosphorylation cycle of PTP1B. (B) Redox regulation cycle of PTP1B. (C) Participation of the unexpectedly formed sulfenyl amide in the reversible redox control of PTP1B.

glutathione (GSH) to regenerate the thiol, and this process maintains the redox state of the protein (Figure 1B).⁵ The importance of sulfenyl acids in biology is well recognized in recent years, and the diverse role of this species in various redox reactions involving proteins and synthetic compounds has been shown to be extraordinary.^{5–7} Although many enzymes protect their active site cysteines from overoxidation and irreversible inactivation by forming an internal disulfide bond with a second

- (1) (a) Neel, B. G.; Tonks, N. K. *Curr. Opin. Cell Biol.* **1997**, *9*, 193–204. (b) Hunter, T. *Cell* **2000**, *100*, 113–117. (c) Zhang, Z. Y. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 209–234. (d) Tonks, N. K. *Cell* **2005**, *121*, 667–670.
- (2) (a) Johnson, T. O.; Ermoloff, J.; Jirousek, M. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 696–709. (b) Hooft van Huijsduijnen, R.; Sauer, W. H. B.; Bombrun, A.; Swinnen, D. J. *Med. Chem.* **2004**, *47*, 4142–4146.
- (3) (a) Denu, J. M.; Tanner, K. G. *Biochemistry* **1998**, *37*, 5633–5642. (b) Barrett, W. C.; DeGnore, J. P.; Keng, Y.-F.; Zhang, Z.-Y.; Yim, M. B.; Chock, P. B. *J. Biol. Chem.* **1999**, *274*, 34543–34546. (c) Chiarugi, P.; Fiaschi, T.; Taddei, M. L.; Talini, D.; Giannoni, E.; Rauegi, G.; Ramponi, G. *J. Biol. Chem.* **2001**, *276*, 33478–33487. (d) Lee, S.-R.; Yang, K.-S.; Kwon, J.; Lee, C.; Jeong, W.; Rhee, S. G. *J. Biol. Chem.* **2002**, *277*, 20336–20342. (e) Sohn, J.; Rudolph, J. *Biochemistry* **2003**, *42*, 10060–10070.

(4) Barford, D. *Curr. Opin. Struct. Biol.* **2004**, *14*, 679–686.

(5) Seth, D.; Rudolph, J. *Biochemistry* **2006**, *45*, 8476–8487, and references therein.

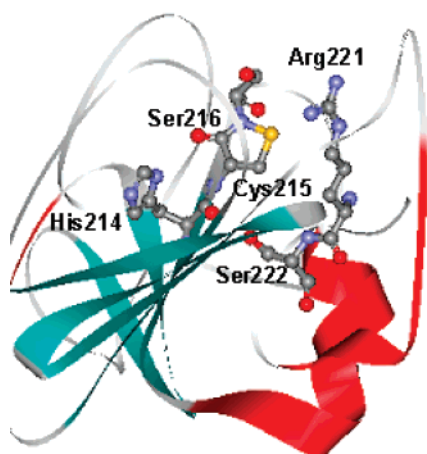


Figure 2. A part of the crystal structure of PTP1B showing the sulfenyl amide (PDB Code: 1OEM).^{8a}

cysteine residue within the active site or a mixed disulfide bond with external thiols,^{5,6} the sulfenic acid intermediate produced in response to PTP1B oxidation is rapidly converted into a sulfenyl amide species (Figure 2), in which the sulfur atom of the catalytic cysteine is covalently bonded to the main chain nitrogen of an adjacent residue (Figure 1C).⁸ This novel and unusual protein modification has been shown to protect the active site cysteine from irreversible oxidation to sulfinic (E-SO₂H) or sulfonic (E-SO₃H) acids.⁸ Similarly to the disulfide bonds in proteins, the formation of sulfenyl amide is reversible and the cleavage of the S–N bond by cellular thiols such as glutathione (GSH) converts the inactivated protein back to its catalytically active form. Furthermore, the inactivated PTP1B can also be reactivated enzymatically by cysteine-containing redox proteins such as thioredoxin or glutaredoxin.⁵

Recently, the conversion of a benzanilide-derived sulfenic acid (**3**), generated by thermolysis of the corresponding β -sulfinyl propionic acid ester, to the corresponding 3-isothiazolidinone (**5**) has been shown to be a chemical model for the cyclization reaction.⁹ Although the nitrogen atoms of amides are generally considered poor nucleophiles due to the conjugation of the amide nitrogen with the carbonyl group,^{9,10} this interesting model study suggested that the sulfenic acid residue may have sufficient electrophilicity to drive the cyclization with an amide backbone. This model study also suggested that the further conversion of the sulfenic acid to a sulfenyl peroxide or a mixed disulfide may not be required for the oxidative transformation of PTP1B

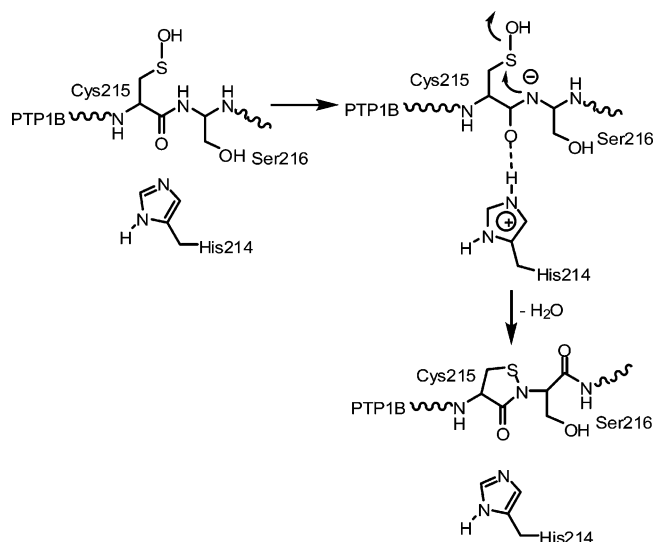


Figure 3. Proposed mechanism showing the participation of His214 in the cyclization of sulfenic acid state of PTP1B to the sulfenyl amide.

to its inactive 3-isothiazolidinone form. During our attempts to mimic the action of PTP1B with synthetic models, we observed that the cyclization of aromatic sulfenic acids bearing mono-substituted *ortho*-amide substituents to the corresponding sulfenyl amides requires a very large activation energy, which can be brought down dramatically by introducing an additional substituent having heteroatoms at the 6-position that can interact with the sulfur atom. Therefore, it is important to consider the steric and electronic effects around sulfur¹¹ in PTP1B for designing synthetic models for the active site of the enzyme. In this paper, we describe an excellent model for the redox regulation of PTP1B and show the first example of how the S \cdots O/N nonbonded interactions involving sulfur and other heteroatoms in the sulfenic acid intermediate modulate the cyclization process. We also show that the formation of a sulfenyl amide species occurs even in the presence of thiols when the electrophilicity of the sulfur in the sulfenic acid intermediate is enhanced by S \cdots N interactions and the –S–OH group of the sulfenic acid adopts a strained conformation.

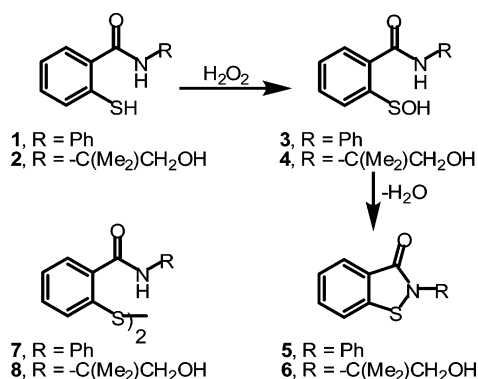
Results and Discussion

As mentioned in the introduction, the oxidation of cysteine residue in PTP1B by H₂O₂ produces the corresponding sulfenic acid intermediate, which undergoes an unusual modification to produce a sulfenyl amide species.⁸ In these studies, Barford et al. and Jhoti et al. have independently shown that the protein modification occurs through oxidation of Cys215 to sulfenic acid, followed by a nucleophilic attack of the backbone nitrogen atom of Ser216 on the sulfur atom of Cys215. This cyclization reaction has been shown to be facilitated by a hydrogen bonding between the carbonyl oxygen atom of Cys215 and N δ 1 atom of the His214, which increases the partial charge on the backbone nitrogen atom of Ser216 (Figure 3).⁸

In our study, we first evaluated the conversion of the benzanilide-derived sulfenic acid (**3**) to the corresponding 3-isothiazolidinone (**5**) in acetonitrile by using an HPLC method.

- (6) (a) Yeh, J. I.; Claiborne, A.; Hol, W. G. *J. Biochemistry* **1996**, *35*, 9951–9957. (b) Giles, G. I.; Jacob, C. *Biol. Chem.* **2002**, *383*, 375–388. (c) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4742–4758. (d) Noguchi, T.; Nojiri, M.; Takei, K.; Odaka, M.; Kamiya, N. *Biochemistry* **2003**, *42*, 11642–11650. (e) Jacob, C.; Holme, A. L.; Fry, F. H. *Org. Biomol. Chem.* **2004**, *2*, 1953–1956.
- (7) (a) Nakamura, N. *J. Am. Chem. Soc.* **1983**, *105*, 7172–7173. (b) Goto, K.; Tokitoh, N.; Okazaki, R. *Angew. Chem., Int. Ed.* **1995**, *34*, 1124–1126. (c) Saiki, T.; Goto, K.; Tokitoh, N.; Okazaki, R. *J. Org. Chem.* **1996**, *61*, 2924–2925. (d) Ishii, A.; Komiya, K.; Nakayama, J. *J. Am. Chem. Soc.* **1996**, *118*, 12836–12837. (e) Goto, K.; Holler, M.; Okazaki, R. *J. Am. Chem. Soc.* **1997**, *119*, 1460–1461. (f) Okazaki, R.; Goto, K. *Heteroat. Chem.* **2002**, *13*, 414–418. (g) Goto, K.; Shimada, K.; Nagahama, M.; Okazaki, R.; Kawashima, T. *Chem. Lett.* **2003**, *32*, 1080–1081. (h) Goto, K.; Shimada, K.; Furukawa, S.; Miyasaka, S.; Takahashi, Y.; Kawashima, T. *Chem. Lett.* **2006**, *35*, 862–863.
- (8) (a) Salmee, A.; Anderson, J. N.; Myers, M. P.; Meng, T.-C.; Hinks, J. A.; Tonks, N. K.; Barford, D. *Nature* **2003**, *423*, 769–773. (b) van Montfort, R. L. M.; Congreve, M.; Tisi, D.; Carr, R.; Jhoti, H. *Nature* **2003**, *423*, 773–777.
- (9) Sivaramakrishnan, S.; Keerthi, K.; Gates, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 10830–10831.
- (10) Gajda, T.; Zwierzak, A. *Synthesis* **1981**, 1005–1008.

- (11) (a) Steric and electronic factors have been shown to play important roles in the stability of sulfenic acids. Davis, F. A.; Jenkins, L. A.; Billmers, R. L. *J. Org. Chem.* **1986**, *51*, 1033–1040. (b) For an excellent article on sulfenic acids and sulfur–nitrogen compounds, see: Davis, F. A. *J. Org. Chem.* **2006**, *71*, 8993–9003 and references therein.

Scheme 1. Conversion of Thiols to the Corresponding Sulfenyl Amides in the Presence of H_2O_2 **Table 1.** Initial Rates (v_0) for the Cyclization of **1** and **2** by H_2O_2 at 23 °C^a

entry	substrate	initial rate, v_0 ($\mu\text{M}\cdot\text{min}^{-1}$)
1	1	0.056
2	1 + H_2O_2	0.118
3	2	0.015
4	2 + H_2O_2	0.124

^a The rates were calculated by following the initial formation of the sulfenyl amides using a reversed-phase HPLC method. Conditions: [**1** or **2**] = 0.17 mM, [H_2O_2] = 0.45 mM. The reactions were carried out in MeCN.

The addition of H_2O_2 to thiol **1** produced the cyclic compound **5**, presumably *via* the sulfenic acid **3** derivative (Scheme 1).⁹ In agreement with the previous report,⁹ the sulfenic acid **3** could not be isolated due to the instability of this species in solution. However, the reaction of **1** with H_2O_2 to produce the sulfenyl amide **5** was found to be extremely slow (Table 1). On the other hand, the reaction of **1** with H_2O_2 in acetonitrile afforded the disulfide **7** as the major product. The identity of **7** was confirmed by comparing the NMR data and HPLC profiles for the major product of this reaction with that of the authentic sample of **7**. The disulfide was found to be unstable in solution, and this compound underwent a disproportionation reaction¹² to produce the sulfenyl amide **5**. A similar type of cyclization has been previously observed for some cystinyl peptide disulfides, which are oxidized readily by bromine to give the corresponding dipeptide isothiazolidinones.¹³ The disproportionation reaction leading to the formation of **5** is found to be highly solvent dependent. To understand the effect of various solvents on the disproportionation reaction, we have determined the rate of conversion of the disulfide **7** (0.05 mM) to the cyclic compound **5** in water, acetonitrile, and methanol and found that the initial rate for the disproportionation of the disulfide **7** to the sulfenyl amide **5** in acetonitrile ($0.049 \mu\text{M}\cdot\text{min}^{-1}$) is much slower as compared to that in methanol ($0.244 \mu\text{M}\cdot\text{min}^{-1}$) and water ($0.094 \mu\text{M}\cdot\text{min}^{-1}$). This is in accordance with the observation

of Domagala et al. that 2,2'-dithiobisbenzamides exit in equilibrium with the corresponding 1,2-beniso-thiazolin-3(2*H*)-ones in solution.¹⁴ Therefore, the conversion of **1** to the sulfenyl amide **5** by reaction with H_2O_2 was studied in acetonitrile to avoid the possible conversion of the disulfide (**7**) to compound **5**. In this case, the reaction sequence involving oxidation of the thiol moiety followed by cyclization of the resulting sulfenic acid was found to be extremely slow and the disulfide was isolated from the reaction in nearly quantitative yield, supporting the assumption that the disulfide **7** is the major precursor for compound **5**. The yield of the disulfide **7** was unaffected when the reaction was performed in the presence of an excess amount of methyl iodide in acetonitrile. Although methyl iodide blocks the potential dimerization of the sulfenic acid **3** to the corresponding thiosulfinate ($\text{RS}(\text{O})\text{SR}$),⁹ this does not block the reaction between the thiol **1** and the sulfenic acid **3** to produce the disulfide **7**.¹⁵ Interestingly, the sulfenic acid **3** does not produce the disulfide **7** when it is generated by a route that does not involve a thiol species, e.g., the thermolysis of the corresponding β -sulfinyl propionic acid ester.⁹ It should be mentioned that the oxidation of PTP1B-SH does not produce any disulfide species and the formation of a protein-bound sulfenyl amide from the thiol, therefore, should occur through the generation of the corresponding sulfenic acid as shown in Figure 1.

To understand the effect various substituents on the cyclization, we have chosen to study compound **2** having an alcoholic moiety attached to the amide side chain. Our initial assumption was that the alcoholic group in **2** might mimic the structural features of the serine residue (Ser216, Figure 3) attached to the nitrogen atom of the amide backbone in PTP1B. Similarly to the oxidation of **1**, the addition of H_2O_2 to **2** produced the cyclic compound **6**, probably through the formation of the sulfenic acid intermediate (**4**). As in the case of **1**, the sulfenic acid derived from **2** could not be isolated due to its instability in solution and possible conversion to other oxidized species such as sulfinic and sulfonic acids. However, the rate of cyclization of **2** in the presence of H_2O_2 was found to be identical with that of **1** (Table 1), indicating that the replacement of the phenyl group in **1** by an alcoholic moiety does not have any significant effect on the cyclization process. In agreement with our observations on compound **1**, the NMR and HPLC studies indicated the formation of disulfide **8** as the major product, which disproportionated in solution to afford the sulfenyl amide **6** as a stable product. The disulfide **8** was isolated and characterized by NMR and mass spectral techniques. The single-crystal X-ray structure of compound **8** reveals unusual $\text{S}\cdots\text{O}$ noncovalent interactions between the sulfur and amide backbone, which probably facilitate the disproportionation reaction in solution.

To further understand the reason for the poor reaction rates observed for the cyclization of **3** and **4** to generate the corresponding sulfenyl amides, we carried out density functional theory (DFT) calculations using the B3LYP/6-31G(d) level of theory (see Supporting Information for all optimized geom-

(12) The disproportionation of the disulfide **7** in phosphate buffer (0.25 M, pH 7.5 at 25 °C) affords a 1:1 mixture of the sulfenyl amide **5** and the thiol **1**. In the presence of air and H_2O_2 , the thiol produced in this reaction undergoes an auto-oxidation and H_2O_2 -mediated oxidation, respectively, to reproduce the disulfide, and this process continues until most of the thiol/disulfide mixture is converted to the sulfenyl amide **5**. It should be noted that the formation of the disulfide **7** in the H_2O_2 -mediated oxidation of **1** is mainly due to the reaction between the thiol **1** and the sulfenic acid **3** and not due to the dimerization of the sulfenic acid. The formation of compounds **1** and **5** in the disproportionation of **7** was followed by HPLC, and the products were compared with authentic samples.

(13) Shiau, T. P.; Erlanson, D. A.; Gordon, E. M. *Org. Lett.* **2006**, *8*, 5697–5699.

(14) Domagala, J. M.; Bader, J. P.; Gogliotti, R. D.; Sanchez, J. P.; Stier, M. A.; Song, Y.; Prasad, J. V. N. V.; Tummino, P. J.; Scholten, J.; Harvey, P.; Holler, T.; Gracheck, S.; Hupe, D.; Rice, W. G.; Schultz, R. *Bioorg. Med. Chem.* **1997**, *5*, 569–579.

(15) For involvement of sulfenic acids in the oxidation of thiols to produce disulfides, see: Davis, F. A.; Billmers, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 7016–7018.

eties). These calculations indicated the presence of strong intramolecular $S\cdots O$ interactions in the sulfenic acids **3** and **4** with $S\cdots O$ distances of 2.46 and 2.45 Å, respectively, which are much shorter than the sum of the van der Waals radii of sulfur and oxygen atoms (3.25 Å). These interactions force the $-OH$ group to adapt a position *trans* to the $S\cdots O$ interaction, leading to an almost linear arrangement of the $O\cdots S-O$ moiety (bond angle: 176.6° for **3** and 176.8° for **4**). As a result of this unusual arrangement, the cyclization partners, i.e., the $-NH-$ and $-OH$ functionalities are positioned in opposite directions, which may hamper a facile cyclization involving the $-NH-$ and $-OH$ groups. Further insights into the relative rate of cyclization of the sulfenic acids were obtained from the DFT calculations by comparing the activation energy profiles for the conversion of the sulfenic acids to the corresponding sulfenyl amides. These calculations suggest that the cyclization of a sulfenic acid having an amide moiety in the *ortho* position needs to overcome several energy barriers before its possible conversion to the sulfenyl amide. The major steps for the cyclization of sulfenic acids include the following: (i) breaking of the intramolecular $S\cdots O$ nonbonding interaction and rotation of the amide $-NH-$ group toward the sulfur atom, (ii) rotation of the $-OH$ group toward the $-NH-$ group of the amide moiety, and (iii) elimination of a water molecule to form the expected cyclic sulfenyl amide. The optimized geometries of various intermediates and transition states (TS) involved in the cyclization reaction of **4** are summarized in Figure 4. The relative electronic energies (including the ZPVE correction) in the gas phase calculated at the B3LYP/6-31G(d) level of theory for the cyclization of **4** are summarized in Table 2, and the corresponding energy profile is shown in Figure 5.

In the first step of conversion of **4** to **6**, it is assumed that the amide nitrogen atom moves toward the sulfur atom by breaking the $S\cdots O$ nonbonded interaction to form **4a**. The conversion of **4** to **4a** through the transition state **4TSI** involves weakening of the $S\cdots O$ nonbonded interaction, which leads to an increase in the energy by 4.6 kcal/mol. This conversion also brings down the distance between sulfur and the amide nitrogen atom from 4.36 Å to 2.94 Å. The energy for the **4TSI** was calculated to be 7.76 kcal/mol higher than that of **4**. In the second step, the hydroxyl group moves toward the amide nitrogen via **4TSII**, leading to the formation of **4b**, which is only 0.67 kcal/mol more stable than the sulfenic acid **4a**. The conversion of **4b** to **4c** is found to be the rate-determining step for the overall process. The closer approach of the $-OH$ group toward the amide nitrogen leads to the elimination of a water molecule. At this stage, the formation of a hydrogen bonding was observed between the sulfur atom and the $-NH-$ group with a $N-H\cdots S$ distance of 2.42 Å. The conversion of **4b** to **4c** takes place via **4TSIII**, for which the energy was calculated to be 49.29 kcal/mol higher than that of **4b**. The transition state **4TSIII** is raised very high in energy and the formation of a water molecule is almost complete as evidenced by a very short $O-H$ distance of 1.03 Å, which is very close to that of a covalent $O-H$ bond. The most interesting feature in **4TSIII** (see Figure 4) is that the nitrogen atom keeps itself away from the sulfur atom ($N\cdots S = 2.68$ Å) with a 15.2° deviation from the aryl- $C-S$ plane. In the structure **4c**, the water molecule is bound to the product via two strong hydrogen bonds. Interestingly, the hydrogen bonds appear to stabilize the sulfenyl amide. There-

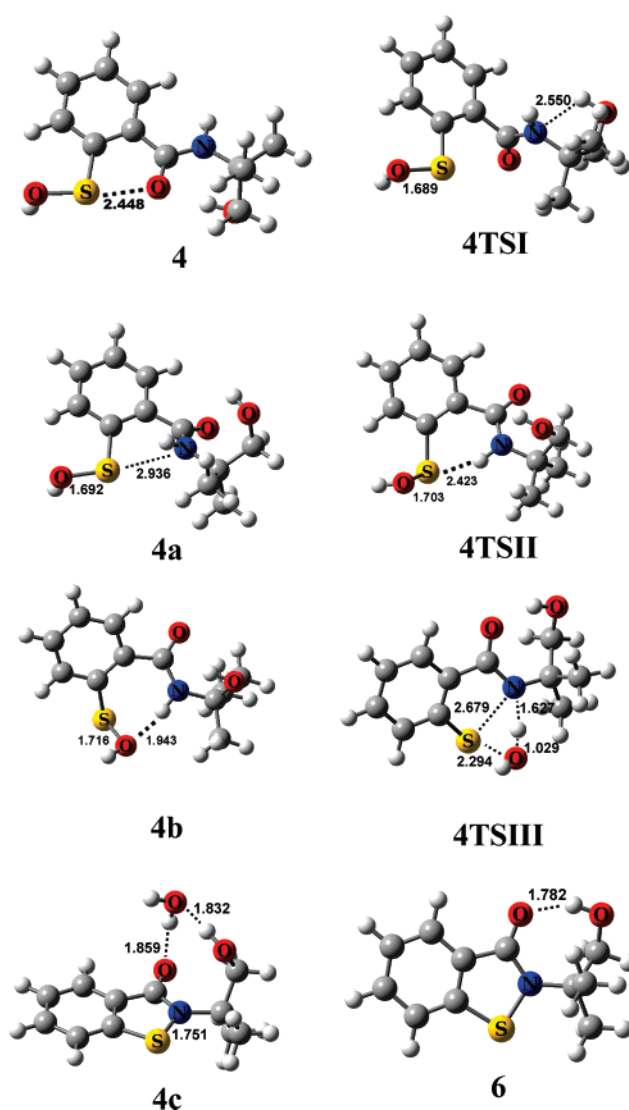


Figure 4. B3LYP/6-31G(d) level optimized geometries of different intermediates and transition states involved in the cyclization of the sulfenic acid **4** to the cyclic sulfenyl amide **6**.

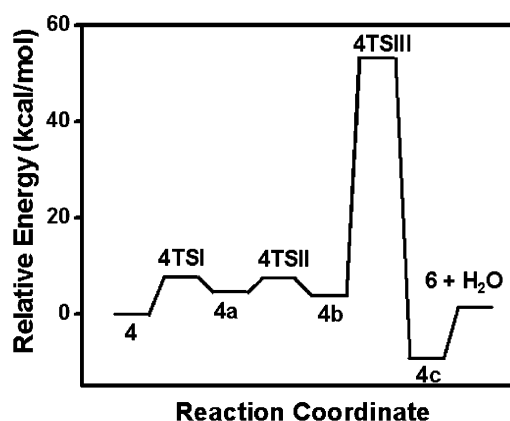


Figure 5. Energy ($\Delta E_{\text{elec}} + \text{ZPVE}$) profiles for the cyclization of **4** to the corresponding sulfenyl amide **6**.

fore, the removal of the water molecule to form the final product **6** is found to be an endothermic process by 10.7 kcal/mol.

These observations reveal that some of the amino acid residues in the active site of PTP1B may facilitate the cyclization by providing steric and/or electronic effects. As shown in Figure

Table 2. Relative Electronic $\Delta(E + \text{ZPE})$ and Relative Gibbs Free Energy $\Delta(G + \text{ZPE})$ for the Cyclization of Sulfenic Acid **4** to the Corresponding Sulfenyl Amides **6** Calculated at the B3LYP/6-31G(d) Level of Theory

		4 \rightarrow 6 + H ₂ O						
	4	4TSI	4a	4TSII	4b	4TSIII	4c	6 + H ₂ O
$\Delta(E + \text{ZPE})$	0	7.76	4.60	7.51	3.92	53.21	−9.23	1.47
$\Delta(G + \text{ZPE})$	0	8.02	4.47	7.68	4.36	54.02	−8.57	−7.21

3, the hydrogen bond between the carbonyl oxygen atom of Cys215 and the N δ 1 atom of His214 not only increases the partial negative charge on the nitrogen but also may prevent the possible S \cdots O interactions in the sulfenic acid intermediate. A careful analysis of the reported crystal structure of PTP1B–SOH reveals that the carbonyl oxygen atom lies away from the sulfur atom (S \cdots O: 4.518 Å),⁸ confirming the absence of any S \cdots O interactions. The theoretical calculations on sulfenic acids **3** and **4** also suggest that the absence of S \cdots O interactions alone is not sufficient for an effective cyclization, as the sulfenic acid intermediate such as **4b**, in which the S \cdots O interactions are absent, still requires a large activation energy (49.29 kcal·mol^{−1}) to produce the sulfenyl amide. Therefore, the formation of the transition state **4TSIII** from the intermediate **4b** is the most important step in the cyclization process. As a large amount of energy is required to break the S–O bond and to form the planar five-membered isothiazolidinone ring in the transition state **4TSIII**, the introduction of any substituent that can assist the S–O bond cleavage may lower the transition state and enhance the cyclization process.

Our experimental and theoretical studies on the cyclization of **3** and **4** and the analysis of PTP1B active site features suggest that the oxidation of thiols to the corresponding disulfides and the electronic environment around the sulfenic acid moiety (–SOH) need to be taken into account while designing biomimetic models for the unusual protein modification at the active site of PTP1B. In this regard, we synthesized thiol **9**, in which the 6-H in compound **2** has been replaced by an oxazoline moiety. The introduction of this five-membered heterocycle may prevent the S \cdots O interactions between the sulfur atom and the oxygen atom of the amide backbone in the sulfenic acid intermediate. The introduction of steric hindrance in the thiol is expected to prevent the oxidation of the thiol to the corresponding disulfide. Crucially, the possible S \cdots N interactions between the sulfur atom and the five-membered heterocyclic nitrogen atom in the sulfenic acid may force the –OH moiety to approach the –NH– group.¹⁶ Subsequently, this arrangement may facilitate the cyclization reaction, leading to the formation of the expected sulfenyl amide. With these aspects in mind, we treated the thiol **9** with hydrogen peroxide in acetonitrile and followed the reaction by HPLC. Interestingly, this reaction resulted in a quantitative conversion of the thiol **9** to the sulfenyl amide **11** (Scheme 2), indicating that the additional substituent at the 6-position plays an important role in the cyclization reaction. This is in contrast to thiols **1** and **2**,

(16) In contrast to the S \cdots O interactions that are well described in the literature, compounds having nonbonded S \cdots N interactions are extremely rare. For some well-defined examples of nonbonded interactions between divalent sulfur and nitrogen, see: (a) Chivers, T.; McGarvey, B.; Parvez, M.; Vargas-Baca, I.; Ziegler, T. *Inorg. Chem.* **1996**, *35*, 3839–3847. (b) Chivers, T.; Krouse, I.; Parvez, M.; Vargas-Baca, I.; Ziegler, T.; Zoricak, P. *Inorg. Chem.* **1996**, *35*, 5836–5842. (c) Mugesh, G.; Singh, H. B.; Butcher, R. J. *Eur. J. Inorg. Chem.* **1999**, 1229–1236. (d) Iwaoka, M.; Takemoto, S.; Okada, M.; Tomoda, S. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1611–1625.

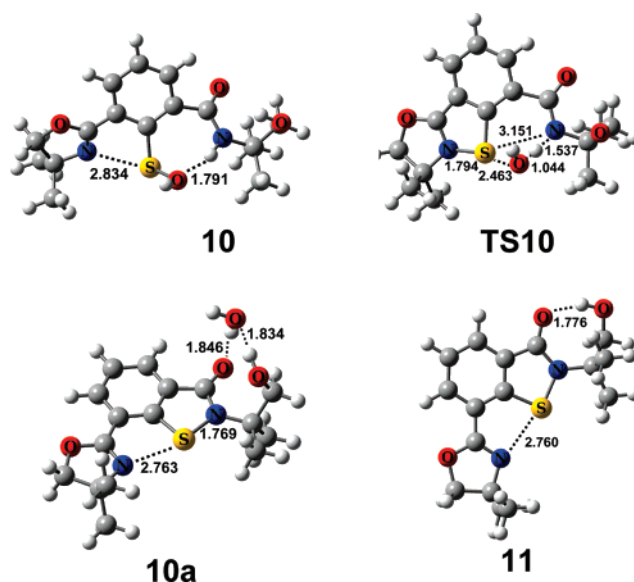
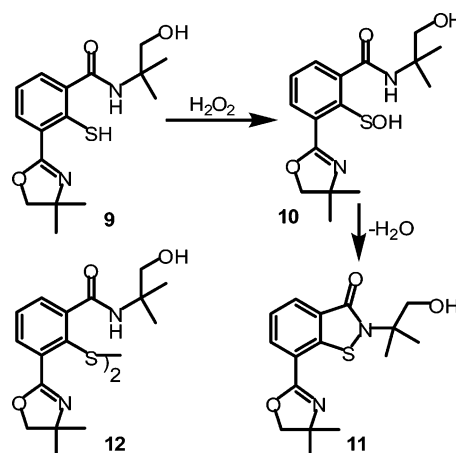


Figure 6. B3LYP/6-31G(d) level optimized geometries of different intermediates and transition states involved in the cyclization of the sulfenic acid **10** to the cyclic sulfenyl amide **11**.

Scheme 2. Proposed Mechanism for the Formation of Sulfenyl Amide **11** from Thiol **9**



which produce the corresponding sulfenyl amides in small amounts. As expected, the formation of disulfide **12** was not detected during the entire process, which is in agreement with the PTP1B protein modification that does not produce any disulfide species during the formation of the sulfenyl amide.

The DFT calculations show that the sulfur atom in sulfenic acid **10** is not involved in an interaction with the carbonyl oxygen. This is in sharp contrast to compounds **3** and **4**, which all exhibited strong S \cdots O interactions. The absence of such interactions is, however, not very important in this case as the energy required to break these interactions is expected to be much lower than that of the overall reaction. The most important feature in the sulfenic acid **10** is the interaction of the heterocyclic nitrogen present in the five-membered oxazoline ring with the sulfur atom, which participates in the cyclization process and dramatically lowers the activation energy barrier (Figure 6). This type of neighboring group participation, which is not possible with monosubstituted sulfenic acids such as **3** and **4**, is crucial for the formation of the sulfenyl amide and the facile elimination of a water molecule. The plot of energy vs reaction coordinates indicates that the conversion of **10** to **11** is energetically more

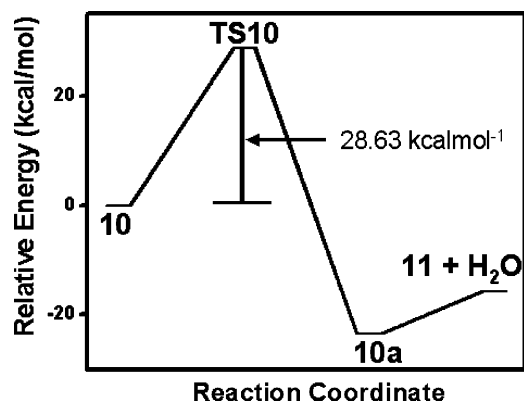
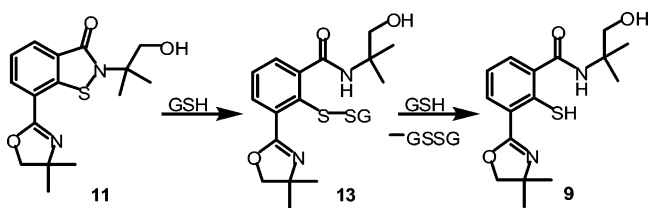


Figure 7. Energy profiles ($\Delta E_{\text{elec}} + \text{ZPVE}$) for the cyclization of **10** to **11**.

Scheme 3. Reduction of Sulfenyl Amide Bond in **11** by GSH.



favored than the conversion of **4** to the sulfenyl amide **6** (Figure 7). These calculations also indicate that the cyclic sulfenyl amide **11** is $15.69 \text{ kcal}\cdot\text{mol}^{-1}$ more stable than the sulfenic acid **10**. The higher stability of the product may also arise from the stabilizing interactions between the heterocyclic nitrogen and the sulfur atoms in compound **11**. The presence of such interactions was unambiguously confirmed by single-crystal X-ray studies on compound **11**, which showed a strong $\text{S}\cdots\text{N}$ interaction ($\text{S}\cdots\text{N}$ distance: 2.760 \AA). The $\text{S}-\text{N}$ covalent bond length of 1.73 \AA in **11** was found to be comparable with that of the sulfenyl amide of PTP1B (1.70 \AA).^{8b}

Despite its higher stability, compound **11** undergoes facile reaction with thiols such as benzene thiol (PhSH), glutathione (GSH), or dithiothreitol (DTT), to produce the sterically hindered thiol **9**. The dithiol DTT was found to be a better reducing agent than the monothiols. These reactions are expected to proceed through the formation of a mixed disulfide (e.g., **13**, Scheme 3) as previously described for **5**.⁹ However, the sulfenyl amide **11** was found to exist in equilibrium with the corresponding thiol (**9**). A large amount of reducing thiol was, therefore, required for the quantitative conversion of **11** to **9**. This is in agreement with the report of Barford et al. that the sulfenic acid derivative of PTP1B undergoes cyclization even in the presence of thiols to produce the corresponding sulfenyl amide.^{8a} Our observations, therefore, support the assumption that the enzyme PTP1B would produce sulfenyl amide species in the reducing environment of the cell.

The detailed DFT investigations to understand the reason for the rapid cyclization of sulfenic acid **10** to the sulfenyl amide **11** led to some interesting observations. We find that the model system based on **9** offers two distinct advantages over the model based on thiol **2** in the cyclization process. (i) The five-membered oxazoline moiety prevents the $\text{S}\cdots\text{O}$ interactions between the sulfur atom and the oxygen atom of the amide backbone in the sulfenic acid intermediate through steric hindrance. As the $-\text{OH}$ moiety would have to approximately

Table 3. Relative Electronic $\Delta(E + \text{ZPE})$ and Relative Gibbs Free Energy $\Delta(G + \text{ZPE})$ for the Cyclization Reaction of **10** Calculated at the B3LYP/6-31G(d) Level of Theory

	10 \rightarrow 11 + H_2O			
	10	TS10	10a	11 + H_2O
$\Delta(E + \text{ZPE})$	0	28.63	-23.40	-15.69
$\Delta(G + \text{ZPE})$	0	30.12	-23.02	-24.20

occupy the position of the 6-substituent, a strain is imposed in the molecule, which leads to the facile cyclization. (ii) The $\text{S}\cdots\text{N}$ interactions between the sulfur atom and the oxazoline nitrogen atom in the sulfenic acid brings the $-\text{OH}$ moiety close to the $-\text{NH}-$ group, which may facilitate the cyclization process. In agreement with these assumptions, the DFT calculations on the sulfenic acid **10** clearly indicate that the sulfur atom does not interact with the carbonyl oxygen, but it interacts strongly with the nitrogen atoms present in the five-membered oxazoline ring. The resulting strained molecular conformation of **10** brings the cyclization to a single step process, which is in contrast to the cyclization of **4** that needs to cross three different energy barriers for the conversion. The relative electronic energies (including the ZPVE correction) calculated at the B3LYP/6-31G(d) level of theory for the cyclization of **10** to **11** are summarized in Table 3, and the corresponding plot is shown in Figure 7. These data indicate that the total energy required for the conversion of **10** to **11** is almost half when compared with the energy required for the cyclization of **3** or **4**.

In the sulfenic acid **10**, the $-\text{OH}$ group is very close to the $-\text{NH}-$ group and the hydrogen atom present on the $-\text{NH}-$ is hydrogen bonded to the $-\text{OH}$ group ($\text{HO}\cdots\text{HN}$: 1.79 \AA) (Figure 6). The complete elimination of a water molecule was observed for compound **10**, which leads to the generation of **10a** via transition state **TS10**. As a result, the water molecule is bound to the sulfenyl amide *via* two strong hydrogen bonds. The first one is observed between the amide carbonyl oxygen atom and the hydrogen atom that was previously in the $-\text{SOH}$ moiety (1.85 \AA). The second hydrogen bond is observed between the oxygen atom of the eliminated water molecule and the hydroxyl hydrogen atom of the $-\text{CH}_2\text{OH}$ moiety in the side chain (1.83 \AA). The strong $\text{S}\cdots\text{N}$ interaction between the oxazoline nitrogen and the sulfur atom is retained in this structure as evidenced by a short $\text{S}\cdots\text{N}$ distance (2.76 \AA). The conversion of **10** to **10a** proceeds through the transition state **TS10** for which the energy was calculated to be 28.63 kcal/mol higher than that of **10**. In the transition state **TS10**, the water molecule is almost formed as observed from the short distance (1.04 \AA) between the NH hydrogen atom and the oxygen atom of the OH group, which is very close to the $\text{O}-\text{H}$ covalent bond length. However, the hydrogen atom remains hydrogen bonded to the nitrogen (1.54 \AA). The oxygen atom is placed at a distance of 2.46 \AA from the sulfur atom, which is much longer than the $\text{S}-\text{O}$ covalent bond in **10** (1.72 \AA). In the transition state **TS10**, the amide nitrogen atom is positioned away from that of the sulfur atom (3.15 \AA) and the nitrogen atom is placed at about -19.8° out of the plane of the phenyl ring attached to the sulfur atom. The most interesting feature in **TS10** is that the nitrogen atom of the oxazoline ring is located almost within covalent bonding distance with the sulfur atom ($\text{S}\cdots\text{N} = 1.79 \text{ \AA}$).

Based on the experimental observations and theoretical calculations, we suggest an $\text{S}_{\text{N}}2$ type elimination of the $-\text{OH}$

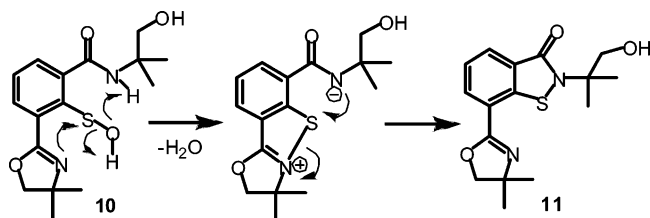
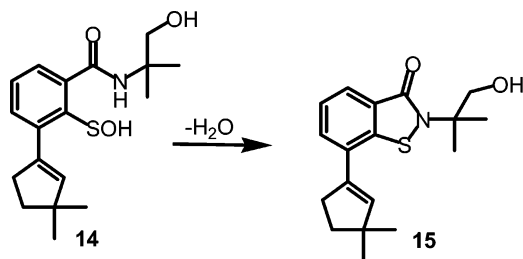


Figure 8. Participation of the oxazoline nitrogen atom in the cyclization of the sulfenic acid **10** to the cyclic sulfenyl amide **11**.

Scheme 4. Effect of Isosteric Replacement of O and N Atoms of Oxazoline Ring in the Cyclization.



group (Figure 8). According to this model, the nucleophilic oxazoline nitrogen atom attacks at the electrophilic sulfur center from the backside. As a result, the —OH group is eliminated as a water molecule by abstracting the required proton from the —NH— group. Consequently, the deprotonation leads to the formation of a highly nucleophilic nitrogen on the amide moiety. The simultaneous attack of this negatively charged nitrogen moiety at the sulfur center and the cleavage of the S—N bond between the sulfur and the oxazoline nitrogen lead to the formation of sulfenyl amide **10a**. The entire process can be viewed as a neighboring group participation of the oxazoline nitrogen followed by two successive elimination reactions (Figure 8). The participation of the neighboring group brings down the barrier for cyclization. The energy barrier for the cyclization of **10** to **10a** was calculated to be $28.63 \text{ kcal}\cdot\text{mol}^{-1}$, which is much lower than that for the conversion of **4** to **6** ($49.29 \text{ kcal}\cdot\text{mol}^{-1}$). In the final step, the departure of the water molecule from **10a** to produce the final compound **11** is found to be an endothermic process by $7.71 \text{ kcal}\cdot\text{mol}^{-1}$.

The importance of the nitrogen in the five-membered oxazoline ring in **10** during an efficient cyclization process was further verified by isosteric replacements of the nitrogen and oxygen atoms in **10** with —CH— and $\text{—CH}_2\text{—}$ groups, respectively (compound **14**). These replacements are expected to retain the steric hindrance at the 6-position but alter the electronic properties of the sulfur. As expected, our theoretical calculations show that the introduction of the five-membered ring having no heteroatoms does maintain the steric bulkiness of the 6-substituent in the sulfenic acid and this steric hindrance is sufficient to prevent any nonbonded $\text{S}\cdots\text{O}$ interactions between the sulfur and carbonyl oxygen in compound **14** (Scheme 4). However, the activation energy barrier calculated for the conversion of the sulfenic acid **14** to the sulfenyl amide **15** ($45.77 \text{ kcal}\cdot\text{mol}^{-1}$, Figure 9) was found to be much higher than that of the conversion of **9** to **11** (28.63 kcal/mol) but comparable with that of the conversion of **4** to **6** (49.29 kcal/mol). These observations clearly suggest that the oxazoline nitrogen participation, which assists the S—O bond cleavage to lower the level of the TS, is more important than the $\text{S}\cdots\text{O}=\text{C}<$ nonbonded interactions.¹⁷ These observations also suggest

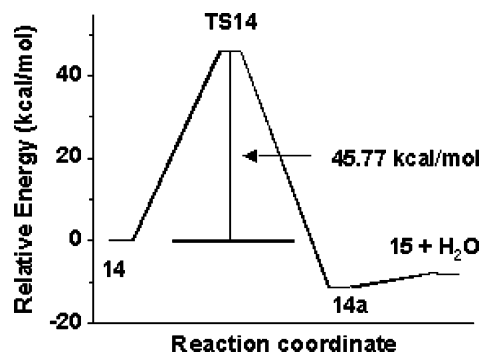


Figure 9. Energy profiles ($\Delta E_{\text{elec}} + \text{ZPVE}$) for the cyclization of **14** to **15**.

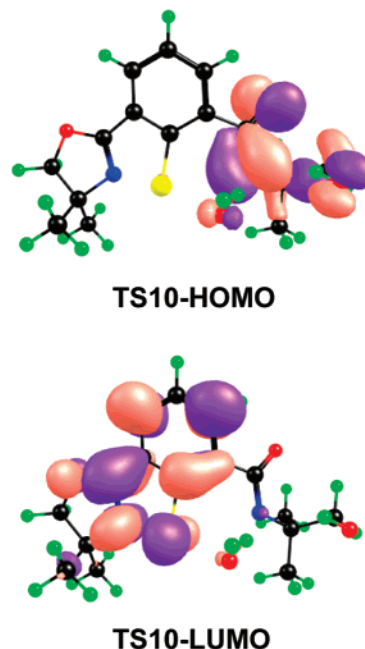


Figure 10. HOMO and LUMO of **TS10** calculated at the B3LYP/6-31G(d) level of theory.

that the introduction of sterically bulky groups that can enforce the proximity of —NH— and —OH— groups is not sufficient for a rapid cyclization of sulfenic acids to sulfenyl amides.

To gain better insight into the role of the oxazoline nitrogen atom during the cyclization reaction, we have obtained the HOMO and LUMO of **TS10** by using the B3LYP/6-31G(d) level of theory (Figure 10). The HOMO represents an orbital delocalized over the whole amide moiety, whereas the LUMO is generated by factors from both the phenyl and the oxazoline rings. Furthermore, the LUMO shows contribution from a π^* (S—N) orbital. The electrons are expected to flow from the HOMO to the π^* (S—N) orbital, which would lead to the cleavage of the S—N (oxazoline ring) bond and the formation of a new S—N (amide) bond leading to the formation **11**. Therefore, the conversion of the sulfenic acid **10** to the sulfenyl amide **11** is both kinetically as well as thermodynamically more favored than the conversion of the sulfenic acid **4** to the

(17) Although simple sulfenic acids can act as either electrophiles or nucleophiles, the sulfenic acids **4**, **10**, and **14** in the present study act as electrophiles towards amide functionality. The NBO charges on the sulfur atoms of these compounds suggest that the sulfur atom in sulfenic acid **10** is more electrophilic than that of **4** and **14**. This is due to the involvement of oxazoline nitrogen in compound **10**. This is also responsible for the weakening of the S—O bond in the **TS10**.

corresponding sulfenyl amide (**6**). Owing to the presence of a strong S \cdots N interaction in **11** and the steric crowding in **10**, compound **11** is 15.69 kcal/mol more stable than the sulfenic acid **10**. On the other hand, the presence of S \cdots O interaction in **4** and the absence of such interactions in **6** indicate the lower thermodynamic stability of the product sulfenyl amide (**6**) compared with the starting sulfenic acid **4**. The HOMO–LUMO calculations confirm our previous observations that the chelating oxazoline ring leads to the stabilization of the transition state (**TS10**) and, therefore, the barrier for cyclization is only half of that calculated for the sulfenic acid **4**.

The oxidation of thiol moieties in **1**, **2**, and **9** by hydrogen peroxide and the facile reduction of sulfenyl amides **5**, **6**, and **11** by thiols prompted us to investigate the antioxidant activity of the sulfenyl amides. The reduction of H₂O₂ was studied by using PhSH as a thiol cosubstrate. The conversion of PhSH to PhSSPh was followed by reversed-phase HPLC, using methanol–water as the eluent. Although the thiols **1**, **2**, and **9** react readily with H₂O₂ to produce the corresponding sulfenic acids, the sulfenyl amides **5**, **6**, and **11** exhibited poor antioxidant activity in the presence of PhSH. A detailed study on the antioxidant behavior and catalytic chemistry of the sulfenyl amides is under investigation.

Conclusions

In the present study, biomimetic models were developed for the unusual formation of a sulfenyl amide species from the sulfenic acid intermediate at the active site of PTP1B. This study reveals that the aromatic sulfenic acids having a monosubstituted amide backbone need to cross several energy barriers for the possible conversion to the corresponding sulfenyl amides. The introduction of an additional substituent at the 6-position in a benzamide-derived sulfenic acid enhances the cyclization process. The introduction of a sterically bulky substituent that can force the –OH group to approach the backbone –NH– moiety and a heteroatom that can interact with the sulfur in the sulfenic acid intermediate to enhance the electrophilicity of sulfur lead to the formation of a sulfenyl amide even in the presence of thiols, supporting the assumption that the enzyme PTP1B would produce the sulfenyl amide species in the reducing environment of the cell. Although the thiol groups in the synthetic models are readily oxidized by hydrogen peroxide and reduced back by thiols, the sulfenyl amides possess a weak antioxidant activity. This is in accordance with the fact that the major function of phosphatases such as PTP1B is not associated with the antioxidant activity.

Experimental Section

General Procedure: *n*-Butyllithium was purchased from Acros Chemical Co. (Belgium). Sulfur powder, sodium borohydride (NaBH₄), and benzanilide were obtained from local suppliers. Methanol was obtained from Merck and dried before use. All other chemicals were of the highest purity available. All experiments were carried out under dry and oxygen-free nitrogen using standard Schlenk techniques for the synthesis. Due to unpleasant odors of several of the reaction mixtures involved, most manipulations were carried out in a well-ventilated fume hood. Mass spectral (MS) studies were carried out on a Q-TOF Micro mass spectrometer with electrospray ionization MS mode analysis. In the case of isotopic patterns, the value given is for the most intense peak. Elemental analyses were performed on a ThermoFinnigan FLASH EA 1112 CHNS analyzer. Liquid-state NMR spectra were recorded in CDCl₃, *d*₄-MeOH, or *d*₆-DMSO as solvent. ¹H (400 MHz) and ¹³C (100

MHz) NMR spectra were obtained on a Bruker Avance 400 NMR spectrometer using the solvent as an internal standard for ¹H and ¹³C. Chemical shifts (¹H, ¹³C) are cited with respect to tetramethylsilane (TMS). Thin-layer chromatography analyses were carried out on precoated silica gel plates (Merck), and spots were visualized by UV irradiation. Column chromatography was performed on glass columns loaded with silica gel or on an automated flash chromatography system (Biotage) by using preloaded silica cartridges. High performance liquid chromatography (HPLC) experiments were carried out on a Waters Alliance System (Milford, MA) consisting of a 2690 separation module, a 2690 photodiode-array detector, and a fraction collector. The assays were performed in 1.8 mL sample vials, and a built-in autosampler was used for sample injection. The Alliance HPLC System was controlled with EMPOWER software (Waters Corporation, Milford, MA). Anthranilic acid was first diazotized and was added to disodium disulfide to produce dithiosalicilic acid, which was treated with freshly distilled thionyl chloride to produce the 2-(chlorothio)benzoyl chloride as a yellow solid. The cyclic sulfenyl amide **6** was prepared by treating 2-(chlorothio)benzoyl chloride with 2-amino-2-methylpropan-1-ol in dry acetonitrile.

Synthesis of 7: To a stirred solution of benzanilide (1.0 g, 5.1 mmol) in dry THF (35 mL) under nitrogen at 0 °C was added *n*-butyllithium (6.4 mL, 10.2 mmol, 1.6 M solution in hexane). After 30 min elemental sulfur (0.16 g, 5.1 mmol) was added to the orange-red solution while a brisk stream of nitrogen was passed through the open system. The solid material was almost consumed after 1 h, and the resulting yellowish-green solution was added to a beaker containing K₃Fe(CN)₆ (1.70 g, 5.2 mmol) in water (150 mL). The precipitated material was filtered off to give, after washing with methylene chloride and drying, 1.40 g (60%) of the disulfide. ¹H NMR (DMSO-*d*₆) δ 10.56 (s, 1H), 7.73–7.78 (m, 4H), 7.52 (t, *J* = 7.2 Hz, 1H), 7.36–7.41 (m, 3H), 7.13 (t, *J* = 7.6 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 165.7, 138.9, 136.5, 134.7, 131.5, 128.8, 128.5, 126.4, 126.3, 124.0, 120.1. MS (TOF MS ES⁺) *m/z* 479.0856 [M + Na]⁺.

Synthesis of 1: To a stirred solution of **7** (40.7 mg, 0.088 mmol) in dry methanol (10 mL) was added sodium borohydride (33 mg, 0.88 mmol) in 5 mL of dry methanol. The reaction mixture was stirred for 24 h at 25 °C, and the solvent was evaporated to dryness and dichloromethane was added to the reaction mixture. The dichloromethane solution was then added to a dilute HCl solution, stirred for 30 min, and then extracted with dichloromethane. The combined dichloromethane extracts were then evaporated under reduced pressure to give colorless oil, which was then purified by flash chromatography using petroleum ether/ethyl acetate as eluent to give the expected thiol as a white solid in 90% yield. ¹H NMR (CDCl₃) δ 7.82 (s, 1H), 7.57–7.62 (m, 3H), 7.35–7.39 (m, 3H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.15–7.22 (m, 2H), 4.55 (s, 1H); ¹³C NMR (CDCl₃) δ 165.9, 136.8, 132.7, 132.3, 130.6, 130.3, 128.4, 127.3, 124.7, 124.1, 119.5. MS (TOF MS ES⁺) *m/z* 252.0462 [M + Na]⁺. Anal. Calcd for C₁₃H₁₁NOS : C, 68.09; H, 4.84; N, 6.11. Found: C, 68.24; H, 4.90; N, 6.15.

Synthesis of 5: This compound was prepared by following the literature method with minor modifications.¹⁸ To a stirred solution of **7** (90 mg, 0.2 mmol) in dichloromethane (5 mL) was added bromine (25 μL, excess mmol), and the reaction mixture was stirred for 12 h at 25 °C. Activated basic alumina (670 mg, excess mmol) was then added, and stirring was continued for an additional 3 h. The solvent was then evaporated under reduced pressure, and the resulting material was purified by column chromatography using neutral aluminum oxide to give a white solid in 80% yield. The ¹H and ¹³C NMR spectral data are in accordance with the literature values.¹⁸ ¹H NMR (CDCl₃) δ 8.11 (d, *J* = 8.0 Hz, 1H), 7.58–7.72 (m, 4H), 7.43–7.50 (m, 3H), 7.33 (t, *J* = 7.6 Hz, 1H); ¹³C NMR (CDCl₃) δ 164.1, 139.8, 137.2, 132.3, 129.3, 127.2, 127.0, 125.8, 124.8, 124.6, 120.1; MS (TOF MS ES⁺) *m/z* 250.0389 [M + Na]⁺.

(18) Kamigata, N.; Hashimoto, S.; Kobayashi, M. *Org. Prep. Proc. Int.* **1983**, *15*, 315–319.

Synthesis of 6: To a stirred solution of 2-amino-2-methylpropan-1-ol (480 μ L, 5 mmol) in dry acetonitrile (50 mL) was added dropwise a solution of 2-(chlorothio)benzoyl chloride (1 g, 4.83 mmol) in acetonitrile (15 mL). The reaction mixture was stirred for 5 h at 25 °C, and the resulting precipitate was filtered off. The filtrate was evaporated under reduced pressure to give a colorless oil, which was then purified by column chromatography using petroleum ether/ethyl acetate (5:1) as eluent to give compound **6** as a white solid in 80% yield. ^1H NMR (CDCl_3) δ 7.96 (d, J = 8.0 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 3.95 (s, 2H), 1.61 (s, 6H). ^{13}C NMR (CDCl_3) δ 166.4, 140.1, 131.8, 126.3, 126.0, 125.5, 119.7, 69.6, 63.8, 24.9; MS (TOF MS ES^+) m/z 246.0529 [$\text{M} + \text{Na}$] $^+$.

Synthesis of 8: To a stirred solution of **6** (100 mg, 0.45 mmol) in CH_2Cl_2 (10 mL) was added excess benzenethiol (460 μ L, 4.5 mmol). The reaction mixture was stirred for 48 h at ambient temperature, and the reaction mixture was kept for aerial oxidation for another 48 h. The solvent was then removed in *vacuo*, and the disulfide was then purified by column chromatography by using ethyl acetate/pet ether 1:1 as eluent to give compound **8** as a white solid in 60% yield. ^1H NMR ($\text{MeOH}-d_4$) δ 7.73 (d, J = 8.0 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 7.6 Hz, 1H), 3.69 (s, 2H), 1.40 (s, 6H); ^{13}C NMR ($\text{MeOH}-d_4$) δ 169.2, 136.3, 135.6, 132.3, 131.1, 130.4, 129.7, 127.5, 127.2, 126.7, 126.3, 67.8, 55.8, 22.7; MS (TOF MS ES^+) m/z 471.1411 [$\text{M} + \text{Na}$] $^+$.

Synthesis of 2: To a stirred solution of the disulfide **8** (20.5 mg, 0.045 mmol) in dry methanol (10 mL) was added sodium borohydride (17 mg, 0.45 mmol) in 5 mL of dry methanol. The reaction mixture was stirred for 24 h at 25 °C, and the solvent was evaporated to dryness and dichloromethane was added to the reaction mixture. The dichloromethane solution was then added to a dilute HCl solution, stirred for 30 min, and then extracted with dichloromethane. The combined dichloromethane extracts were then evaporated under reduced pressure to give colorless oil, which was then purified by flash chromatography using petroleum ether/ethyl acetate as eluent to give compound **2** as a white solid in 90% yield. ^1H NMR (CDCl_3) δ 7.42 (d, J = 6.8 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.26 (t, J = 6.8 Hz, 1H), 7.16 (t, J = 6.8 Hz, 1H), 6.07 (b, 1H), 4.40 (s, 1H), 3.72 (s, 2H), 1.41 (s, 6H); ^{13}C NMR (CDCl_3) δ 168.4, 133.2, 130.8, 130.1, 129.7, 126.9, 126.6, 124.5, 69.2, 55.8, 23.6; MS (TOF MS ES^+) m/z 248.0717 [$\text{M} + \text{Na}$] $^+$.

Synthesis of 11: To a stirred solution of 2,6-bis(4,4-dimethyl-2-oxazoline-2-yl)benzene (0.817 g, 3 mmol) in 30 mL of dry benzene were added TMEDA (1.35 mL, 9 mmol) and LDA (4.5 mL, 9.0 mmol). The reaction mixture was stirred for 4 h, and the resulting precipitate was dissolved in dry THF (30 mL). After the solution cooled to -15 °C, elemental sulfur (0.100 g, 3 mmol) was added. The stirring was continued for 12 h at ambient temperature, and then the mixture was kept for 5 days for aerial oxidation by adding THF from time to time. The solution was then extracted with CH_2Cl_2 . The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed in *vacuo* to afford dark oil. The unusually cleaved product **11** was purified by column chromatography as a stable compound on silica gel with petroleum ether/ethyl acetate (1:1) as eluent in 50% yield. The product was recrystallized from chloroform to give colorless crystals. ^1H NMR (CDCl_3) δ 8.09 (d, J = 7.6 Hz, 1H), 7.97 (d, J = 6.8 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 5.58 (b, 1H), 4.22 (s, 2H), 3.94 (d, J = 3.6 Hz, 2H), 1.68 (s, 6H), 1.44 (s, 6H); ^{13}C NMR (CDCl_3) δ 166.2, 160.4, 141.0, 130.1, 128.6, 127.5, 125.2, 119.7, 80.2, 70.5, 68.1, 63.3, 28.6, 25.0; MS (TOF MS ES^+) m/z 321.1295 [$\text{M} + \text{H}$] $^+$. Anal. Calcd for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$: C, 59.98; H, 6.29; N, 8.74; S, 10.01. Found: C, 60.31; H, 6.38; N, 8.87; S, 10.13.

Synthesis of 9: To a stirred solution of **11** (20 mg, 0.062 mmol) in dry THF (5 mL) was added dithiothreitol (DTT) (19 mg, 0.124 mmol), and the reaction mixture was allowed to stir at room temperature under nitrogen for 48 h. The solvent was removed under *vacuo*, and the expected thiol was purified by flash chromatography to give the final

product in 85% yield. ^1H NMR (CDCl_3) δ : 16.10 (b, 1H), 9.26 (s, 1H), 8.13 (d, J = 9.6 Hz, 1H), 7.94 (d, J = 9.6 Hz, 1H), 6.98 (t, J = 8 Hz, 1H), 5.44 (b, 1H), 4.52 (s, 2H), 3.74 (s, 2H), 1.67 (s, 6H), 1.44 (s, 6H); ^{13}C NMR (CDCl_3) δ 170.2, 167.5, 161.4, 137.9, 134.9, 132.2, 118.8, 117.8, 79.4, 69.5, 62.2, 59.4, 55.8, 26.6, 24.6, 23.7, 13.2.

HPLC Assay: In this assay, we employed a mixture containing a 2:1 molar ratio of PhSH and H_2O_2 in dichloromethane/methanol (95:5) at room temperature as our model system. Runs with and without 10 mol % added test compounds were carried out under the same conditions. Periodically, aliquots were removed and the concentrations of the product diphenyl disulfide (PhSSPh) were determined from the detector response, using pure PhSSPh as an external standard. The initial rates (v_0) for the conversion of thiols to the corresponding sulfinyl amides were calculated from the first 5–10% of the reactions, and the amounts of sulfinyl amides formed in these reactions were determined from the calibration plots of authentic sulfinyl amides. The reduction of H_2O_2 by sulfinyl amides in the presence of PhSH was studied by following a similar method using the PhSSPh as an external standard. The amount of disulfide formed during the course of the reaction was calculated from the calibration plot for the standard.

X-ray Crystallography. X-ray crystallographic studies were carried out on a Bruker CCD diffractometer with graphite-monochromatized Mo $\text{K}\alpha$ radiation (λ = 0.710 73 Å) controlled by a Pentium-based PC running on the SMART software package.¹⁹ Single crystals were mounted at room temperature on the ends of glass fibers, and data were collected at room temperature. The structures were solved by direct methods and refined using the SHELXTL software package.²⁰ All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were assigned idealized locations. Empirical absorption corrections were applied to all structures using SADABS.^{21,22}

Crystal data for 6: $\text{C}_{11}\text{H}_{13}\text{NO}_2\text{S}$; M_r = 223.28, monoclinic, space group $P2(1)/c$, a = 10.0765(56) Å, b = 15.6555(69) Å, c = 7.3410(33) Å, β = 111.081(7)°; V = 1080.56(33) Å³, Z = 4, ρ_{calcd} = 1.37 g/cm, Mo $\text{K}\alpha$ radiation (λ = 0.710 73 Å), T = 273(2) K; R_1 = 0.059, wR_2 = 0.181 ($I > 2\sigma(I)$); R_1 = 0.062, wR_2 = 0.187 (all data). **Crystal data for 11:** $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$; M_r = 320.4, triclinic, space group $P\bar{1}$, a = 9.4859(14) Å, b = 9.7695(15) Å, c = 10.9072(17) Å, α = 90.233(2)°; β = 112.003(2)°; γ = 117.918(2)°, V = 807.99(16) Å³, Z = 2, ρ_{calcd} = 1.32 g/cm, Mo $\text{K}\alpha$ radiation (λ = 0.710 73 Å), T = 293(2) K; R_1 = 0.038, wR_2 = 0.113 ($I > 2\sigma(I)$); R_1 = 0.047, wR_2 = 0.120 (all data). The structures were solved by a direct method (SIR-92)²⁰ and refined by full-matrix least-squares procedures on F^2 for all reflections (SHELXL-97).^{21–22}

Computational Methods. All calculations were performed using the Gaussian98 suite of quantum chemical programs.²³ The hybrid Becke 3–Lee–Yang–Parr (B3LYP) exchange correlation functional was applied for DFT calculations.²⁴ Geometries were fully optimized at the B3LYP level of theory using the 6-31G(d) basis sets. Transition states were located using Schlegel's synchronous transit-guided quasi-Newton (STQN) method.^{25,26} Transition states were searched by using the QST3 keyword, and the resultant conformation was optimized using the TS keyword. Furthermore, the transition state and the stable conformers were characterized by the presence or absence of a single imaginary mode. The activation energies are the difference in the zero-point vibrational energy corrected electronic energy between the transition state and the stable conformations.

(19) SMART, version 5.05; Bruker AXS: Madison, WI, 1998.

(20) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Gualardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343–350.

(21) Sheldrick, G. M. SHELX-97. *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473.

(22) Sheldrick, G. M. SHELX-97, Program for the Refinement of Crystal Structures; University of Göttingen: Göttingen, Germany, 1997.

(23) Gaussian98; Gaussian, Inc.: Pittsburgh, PA, 1998. The full reference is given in the Supporting Information.

(24) (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

(25) Gonzalez, C.; Schlegel, H. B. *J. Chem. Phys.* **1989**, *90*, 2154–2161.

(26) Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523–5527.

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Supporting Information Available: Archive entries for all the optimized geometries reported in this paper and full reference of ref 23. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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