Journal of Molecular Structure 1048 (2013) 410–419

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Redox regulation of protein tyrosine phosphatase 1B (PTP1B): Importance of steric and electronic effects on the unusual cyclization of the sulfenic acid intermediate to a sulfenyl amide

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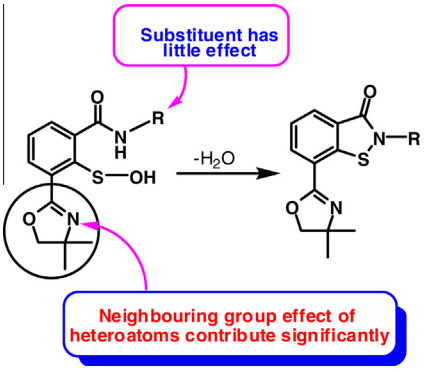
HIGHLIGHTS

- A small molecule chemical model for the redox regultion of PTP1B is described.
- X-ray crystal structure of a sulfoxide obtained by trapping sulfenic acids with CH_3I is reported.
- S···O/N nonbonded interactions play important roles in the cyclization of sulfenic acids.
- Electronic effects may play more important role than steric effects in efficient cyclization of sulfenic acids.
- Other amino acid residues near the active site of PTP1B may assist cyclization of protein sulfenic acid.

GRAPHICAL ABSTRACT

The redox regulation of protein tyrosine phosphatase 1B (PTP1B) via the unusual transformation of its sulfenic acid (PTP1B–SOH) to a cyclic sulfenyl amide intermediate is studied by using small molecule chemical models. These studies show that the substituents that can induce steric environment and alter the electronic properties around the sulfenic acid moiety by S···N or S···O nonbonded interactions can influence the cyclization process. The amino acid residues in the close proximity of the sulfenic acid moiety in PTP1B may play important roles via such interactions in the cyclization of PTP1B–SOH to produce the corresponding sulfenyl amide.



ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 23 April 2013
Accepted 21 May 2013
Available online 30 May 2013

Keywords:
AIM analysis
DFT calculations
NBO analysis
Redox regulation
Sulfenic acid
Sufenyl amide

ABSTRACT

The redox regulation of protein tyrosine phosphatase 1B (PTP1B) via the unusual transformation of its sulfenic acid (PTP1B–SOH) to a cyclic sulfenyl amide intermediate is studied by using small molecule chemical models. These studies suggest that the sulfenic acids derived from the H_2O_2-mediated reactions *o*-amido thiophenols do not efficiently cyclize to sulfenyl amides and the sulfenic acids produced *in situ* can be trapped by using methyl iodide. Theoretical calculations suggest that the most stable conformer of such sulfenic acids are stabilized by $n_O \rightarrow \sigma^*_{S-OH}$ orbital interactions, which force the –OH group to adopt a position *trans* to the S···O interaction, leading to an almost linear arrangement of the O···S–O moiety and this may be the reason for the slow cyclization of such sulfenic acids to their corresponding sulfenyl amides. On the other hand, additional substituents at the 6-position of *o*-amido phenylsulfenic acids that can induce steric environment and alter the electronic properties around the sulfenic acid moiety by S···N or S···O nonbonded interactions destabilize the sulfenic acids by inducing strain in the molecule. This may lead to efficient the cyclization of such sulfenic acids. This model study suggests that the amino

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0022-2860/$ - see front matter
http://dx.doi.org/10.1016/j.molstruc.2013.05.044

acid residues in the close proximity of the sulfenic acid moiety in PTP1B may play an important role in the cyclization of PTP1B–SOH to produce the corresponding sulfenyl amide.

1. Introduction

Protein tyrosine phosphatases (PTPs) are cysteine-containing enzymes, which regulate cellular processes in response to extracellular signals [1]. These enzymes regulate signal transduction pathways involving tyrosine phosphorylation and have been implicated in the development of cancer, diabetes, rheumatoid arthritis and hypertension [2]. PTPs catalyze the dephosphorylation of phosphotyrosine (pTyr) to tyrosine via a phosphocysteine intermediate and together with protein tyrosine kinase modulate the level of protein tyrosine phosphorylation [3]. 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 and metabolism [4]. 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 sulfenic acid (Cys–SOH) [5]. The sulfenic acid reacts with thiols such as glutathione (GSH) to regenerate the thiol, and this process maintains the redox state of the protein (Fig. 1A) [6]. However, the sulfenic acid can undergo further reaction with H_2O_2 to produce overoxidized species such as sulfinic (E-SO_2H) or sulfonic (E-SO_3H) acids. Recently, it has been shown that the sulfenic acid intermediate produced in response to PTP1B oxidation by H_2O_2, is rapidly converted into a sulfenyl amide species, in which the sulfur atom of the catalytic cysteine (Cys215) is covalently bonded to the main chain nitrogen of a serine residue (Ser216) (Fig. 1B) [7]. This novel and unusual protein modification has been shown to protect the active site cysteine from irreversible oxidation to sulfinic (E-SO_2H) or sulfonic (E-SO_3H) acids [7]. Most importantly, the formation of sulfenyl amide is reversible and the cleavage of 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 [6].

In a recent study, Gates and co-workers have shown that the cyclization of the *o*-amido phenylsulfenic acids to their corresponding 3-isothiazolidinones acts as a suitable chemical model for the H_2O_2 mediated cyclization reaction that takes place at the active site of PTP1B (Scheme 1) [8]. We have recently shown that the H_2O_2-mediated cyclization of *o*-amido-thiophenols (**1**–**2**) to form sulfenyl amides (**7**–**8**) occurs due to the disproportionation of the disulfides (**5** and **6**) that are formed from the reaction of

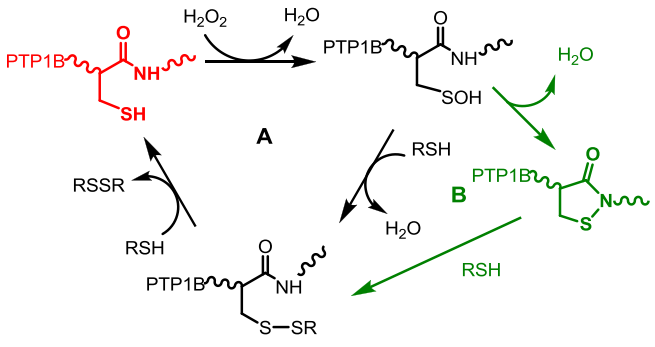
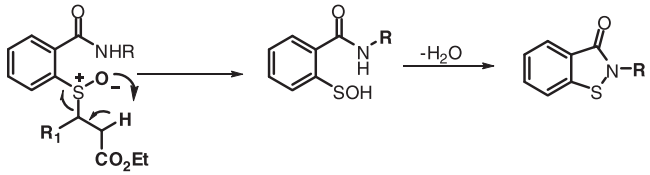


Fig. 1. (A) Redox regulation cycle of PTP1B. (B) Participation of the unexpectedly formed sulfenyl amide in the reversible redox control of PTP1B.



Scheme 1. Cyclization of *in situ* generated sulfenic acid to their corresponding sulfenyl amides.

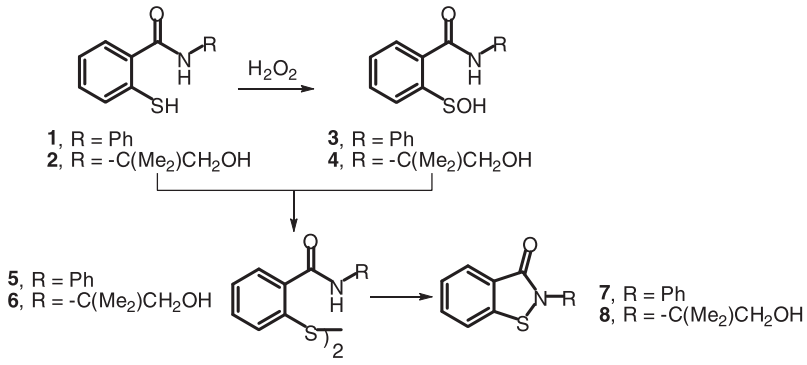
the *in situ* generated sulfenic acids (**3**–**4**) with unreacted thiols (**1**–**2**) (Scheme 2) [9]. Therefore, thiols such as **1** and **2** are not suitable chemical models to mimic the cyclization reaction that takes place at the active site of PTP1B. We have also shown that *o*-amido thiophenol with an additional chelating oxazoline substituent at the 6-position of the phenyl ring (compound **9**) is more efficient and undergoes rapid H_2O_2-mediated cyclization even in the presence of external thiols to form the corresponding sulfenyl amide (**11**) in quantitative yield (Scheme 3) [9]. Therefore, we proposed that it may be important to consider the steric and electronic effects around sulfur [10] for designing suitable chemical models to mimic the cyclization reaction that takes place at the active site of PTP1B.

However, several important questions remained unanswered. For example, (i) how does the *in situ* formed *o*-amido phenylsulfenic acids such as **3** react under different conditions? (ii) Why are the cyclization reactions of *o*-amido phenylsulfenic acids to form sulfenyl amides slow and is it possible to detect or trap such sulfenic acids? (iii) What is the origin of the intramolecular nonbonded S···O interactions in *o*-amido phenylsulfenic acids and what roles do they play in the cyclization reaction? (iv) What role do the substituents on the nitrogen atom of *o*-amido phenylsulfenic acids have in the cyclization reaction? (v) What role do the additional groups such as the oxazoline ring in sulfenic acids **10** play in the cyclization reaction? Is it steric or electronic? (vi) Can the amide groups in the close proximity of protein sulfenic acid (PTP1B–SOH) enhance the cyclization efficiency?

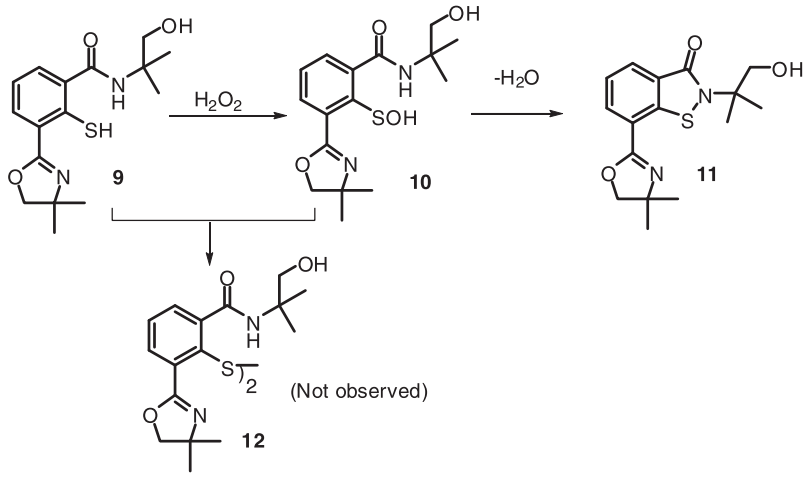
In this paper we studied the following noteworthy features of the cyclization reaction of sulfenic acid to sulfenyl amides:

(i) To determine the fate of the *in situ* produced sulfenic acids, the reaction of thiol **1** with H_2O_2 is studied in detail under different conditions. We observed that the cyclization of the *in situ* produced sulfenic acid **3** is a very slow process and the sulfenic acid **3** can be trapped by using CH_3I. The identity of the sulfoxide (**14**) produced from the reaction of **3** with CH_3I is confirmed by NMR, ESI mass and X-ray crystallographic studies.

(ii) In our previous study, [9] based on the computed distances between the S and the amide O atoms in sulfenic acids **3** and **4**, we predicted that the S and O atoms are involved in intramolecular S···O interactions. We also proposed that due to the intramolecular S···O interaction, the –NH– and –OH functionalities are positioned in opposite directions, which hamper the efficient cyclization reaction of these sulfenic acids to the corresponding sulfenyl amides. We have now studied the nature of this



Scheme 2. Conversion of thiols to the corresponding sulfenyl amides in the presence of H_2O_2.



Scheme 3. Proposed mechanism for the formation of sulfenyl amide **11** from thiol **9**.

interaction and its influence in the cyclization reaction by using Natural Bond Orbital (NBO) [11] and Atoms in Molecules (AIM) [12] analyses using sulfenic acids **3**, **4**, **20** and **21** as models.

(iii) Recently, we showed that sulfenic acid **10** having an oxazoline ring in the *ortho*-position of the sulfur atom cyclizes efficiently and with the help of computational studies we showed that the heterocyclic ring plays an important role in this process by neighboring group participation [9]. Herein, we have studied the role of the oxazoline ring in the cyclization process by isosteric replacement of the ring heteroatoms. We have also evaluated the role of steric effect alone by substituting the oxazoline ring by other bulky groups such as *t*-Bu that lacks heteroatoms. Along with **10**, we have now included sulfenic acids **24**, **26**, **28** and **30** and studied the effect of the additional substituent at the 6- position of o-amido sulfenic acids. These studies show that the steric and electronic effects of heteroatoms near the sulfur center of sulfenic acids may play significant roles in the cyclization process.

(iv) Finally, we have done theoretical calculations on sulfenic acid **32**, as a model, to mimic the possible role of an amide carbonyl group near the sulfenic acid moiety of PTP1B. We propose that the nearby amide oxygen atom can involve in S···O non bonded interactions, sulfenic acid **32** may cyclize more efficiently and, therefore, amido groups near to the sulfenic acid moiety of PTP1B may influence the cyclization process.

2. Results and discussions

2.1. Reaction of thiol *1* with H_2O_2

Recently, we have shown that the cyclization reaction of the *o*-amido thiophenols **1** and **2** in presence of H_2O_2 *via* sulfenic acids **3** and **4** is a very slow process and it is the disproportionation reaction of the disulfides **5** and **6** formed in the reaction that leads to the formation of sulfenyl amides **7** and **8** (Scheme 2) [9]. Further, it was observed that the disproportion reaction of the disulfides **5** and **6** are highly solvent dependent and much slower in CH_3CN compared to protic solvents [9]. Therefore, a detailed analysis of the products formed in the reaction of the *o*-amido thiophenols in presence of H_2O_2 is considered to be of interest to have a better understanding of the cyclization process. Accordingly, we have undertaken a detailed analysis of the products that are formed from the reaction of thiol **1** with H_2O_2 under different conditions.

We have observed that the reaction of thiol **1** with H_2O_2 in CH_3CN produced disulfide **5** in quantitative yield. The yield of disulfide **5** was unaffected when the reaction was carried out in CH_3CN in the presence of an excess amount methyl iodide (CH_3I), (final concentrations: **1**, 17.0 mM; H_2O_2, 20.5 mM; CH_3I, 7.97 M; acetonitrile, ~50% by volume). It is to be noted that the formation of disulfide **5** in the H_2O_2-mediated oxidation of **1** is mainly due to the reaction between the unreacted thiol **1** and the sulfenic acid **3** produced *in situ* and not due to the dimerization of the sulfenic acid as CH_3I is known to block the dimerization of sulfenic acids [8] (Scheme 4). On the other hand, when the reaction of thiol **1** with

H_2O_2 in the presence of an excess amount of CH_3I was carried out in a 1:1 mixture of acetonitrile and phosphate buffer (pH = 7.5) (final concentrations: **1**, 11.9 mM; H_2O_2, 12.0 mM; buffer, 174.6 mM, pH 7.5; CH_3I, 5.58 M; acetonitrile, 30% by volume), we obtained compound **13** (~90%) along with small amounts of sulfoxide **14** (~10%) (Scheme 4) and no disulfide (**5**) formation was observed.

The sulfide **13** is formed due to the fast methylation of thiol **1** in phosphate buffer. This was confirmed by independent reaction of thiol **1** with CH_3I in phosphate buffer, which produced **13** in quantitative yield in 1 h. However, the reaction of thiol **1** with CH_3I was found to be very slow in acetonitrile and **13** was obtained in ~10% yield along with ~30% disulfide **5** (Scheme 5). In this particular case, most of the thiol **1** remained unreacted even after 15 h. Therefore, it is clear that due to the slow reaction between thiol **1** and CH_3I in CH_3CN, the reaction of thiol **1** with H_2O_2 in presence of excess CH_3I produces the disulfide **5** in quantitative yield.

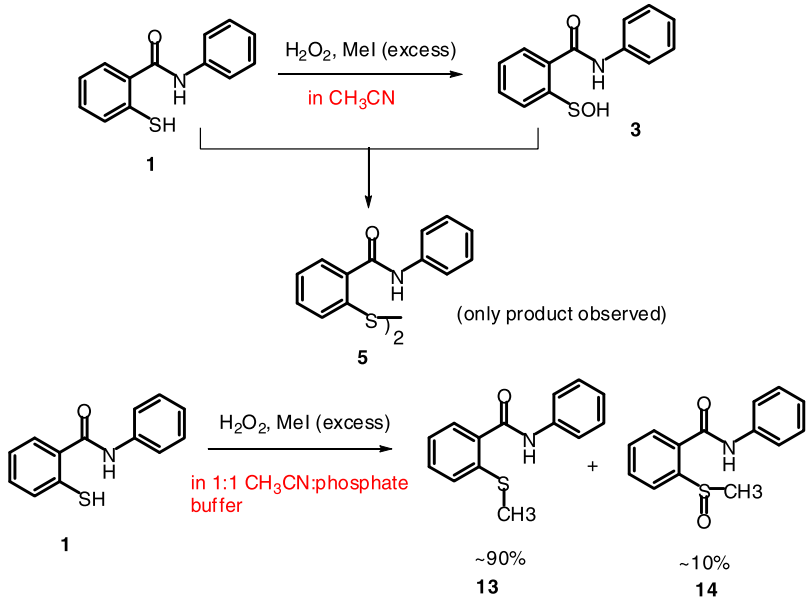
2.2. Disproportionation of the disulfide *5*

We have observed that the disulfide **5** disproportionates to give thiol **1** and sulfenyl amide **7**. Therefore, the yield of sulfenyl amide **7** may depend on the amount of disulfide (**5**) impurity present with thiol **1**. To verify this, we have designed experiments where the disulfide **5** was mixed in different amounts along with a fixed amount of thiol **1.** We observed that the yield of sulfenyl amide **7** increases with an increase in the amount of disulfide **5** added to thiol **1**. In a typical experiment, thiol **1** was mixed with one equivalent of the disulfide **5** and treated with H_2O_2 and CH_3I (excess) in 1:1 a mixture of CH_3CN and phosphate buffer (final concentrations: **1**, 3.97 mM; 5, 3.98 mM; H_2O_2, 13.23 mM; buffer, 174.6 mM, pH 7.5; CH_3I, 5.58 M; acetonitrile, ~35.0% by volume). As expected, we have obtained compounds **7** (35%) and **13** (45%) as major species in the reaction along with sulfoxide **14** in ~10% yield. As discussed above, when a similar reaction of thiol **1** with H_2O_2 in presence of excess of CH_3I was carried out in CH_3CN and phosphate buffer mixture, we only observed the formation of compound **13** and **14** and no sulfenyl amide (**7**) formation was observed. This indicates that the formation of cyclic compound **7** strongly depends on the amount of disulfide (**5**) impurity present in the reaction mixture.

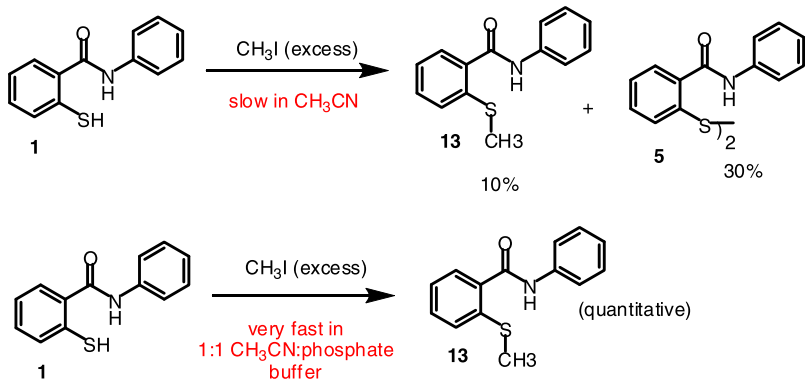
The unusual disproportionation of disulfides **5** and **6** may be because of the presence of S···O interaction as evidenced from the crystal structure of **6**, where both the sulfur atoms are involved in S···O interactions [9]. In contrast to the disulfide **5** and **6**, the unsymmetrical disulfides **15**–**17** (Scheme 6) were found to be stable in solution and did not produce any sulfenyl amide species. This may be because both the sulfur atoms are not involved in S···O interaction in **15**–**17**. DFT calculations using B3LYP/6-31G(d) level of theory [13] reveal the presence of S···O interactions of the sulfur atoms with the *ortho*-amide oxygen atoms in **15** and **17** to form five-membered rings [**15**: $r_{S\cdots O}$ = 2.69 Å; $E_{S\cdots O}$ = 5.29 - kcal mol^{-1}; **17**: $r_{S\cdots O}$ = 2.71 Å; $E_{S\cdots O}$ = 4.71 kcal mol^{-1}] ($E_{S\cdots O}$ = NBO second-order perturbation energy [11]) (see Scheme 6).

2.3. Formation of sulfoxide *14* via methylation of sulfenic acid *3*

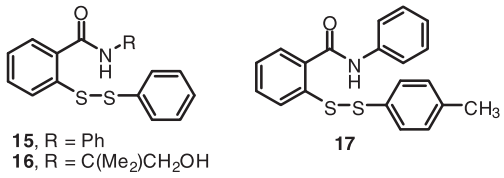
The formation of sulfoxide **14** in the reaction of thiol **1** with H_2O_2 in a 1:1 mixture of acetonitrile and phosphate buffer even in the presence of an excess amount of CH_3I may occur *via* two different pathways: (i) methylation of the sulfenic acid **3** or (ii) oxidation of **13** in presence of H_2O_2. To find out whether the oxidation of **13** by H_2O_2 under the conditions described above can produce **14**, we synthesized compound **13**, purified and treated it with H_2O_2 in CH_3CN/buffer (1:1) (Scheme 7). This reaction, however, did not produce **14** even after 24 h. Similarly, sulfides **18** and **19** that are structurally similar to **13**, did not undergo oxidation in presence of H_2O_2 in CH_3CN/buffer (1:1) even after 24 h. These observations indicate that sulfides such as **13**, **18** and **19** are not reactive to H_2O_2 in CH_3CN/buffer (1:1) under the conditions discussed above. Therefore, the formation of compound **14** must take place via the methylation of the sulfenic acid **3**. The identity of sulfoxide **14** was unambiguously confirmed by NMR, mass and X-ray crystallographic studies (Fig. 2). The crystal structure of **14** shows an unusual S···O interaction between the tetravalent sulfur atom and the amide carbonyl moiety. The S···O distance of 2.75 Å is much shorter than the sum of the van der Waal's radii of sulfur and oxygen atoms (3.25 Å).



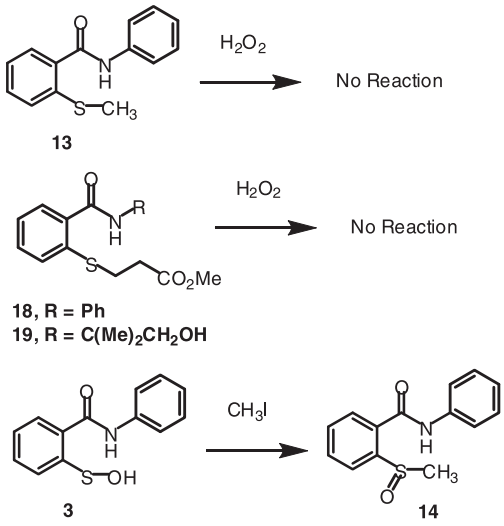
Scheme 4. The reaction of thiol **1** with H_2O_2 in presence of excess of CH_3I produces disulfide **5** in acetonitrile. But the same reaction produces sulfide **13** and sulfoxide **14** in 1:1 acetonitrile:phosphate buffer.



Scheme 5. Reaction of thiol **1** with CH_3I in acetonitrile and 1:1 acetonitrile:phosphate buffer.



Scheme 6. Chemical structures of mixed disulfides **15**–**17**.



Scheme 7. Reaction of sulfenic acid **3** with CH_3I produces sulfoxide **14**. Sulfides **13** and **18**–**19** do not react with H_2O_2.

Interestingly, **14** may lead to the formation of cyclic sulfenyl amide **5** in CH_3CN/buffer because such a transformation is known in the literature for the selenium analogue of **14** [14]. Therefore, we treated **14** with a 1:1 mixture of acetonitrile and phosphate buffer but no sulfenyl amide formation was observed even after 15 h. This indicates that sulfoxide **14** is not involved in the cyclization of **1**–**7** in presence of H_2O_2. These studies suggest that although the oxygen atoms of sulfenic acids are considered as very weak nucleophiles, the nucleophilic attack of oxygen atom of sulfenic acid **3** on CH_3I produces **14**. Therefore, the cyclization of sulfenic acid **3** to sulfenyl amide **7** must be a very slow process and the *o*-amido thiophenols may not be suitable models to study the redox regulation that takes place at the active site of PTP1B.

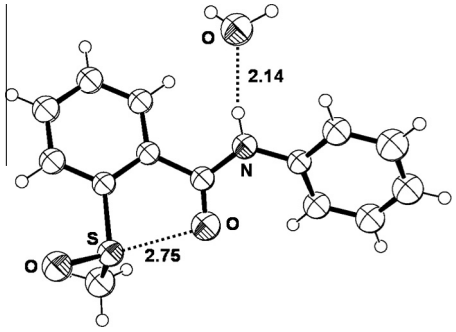


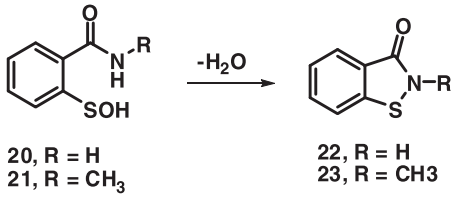
Fig. 2. Crystal structure of **14** shows the presence of S···O nonbonded interaction. A water molecule was found to be hydrogen bonded to the amide –NH– group.

2.4. Theoretical studies

2.4.1. Intramolecular S···O nonbonded interactions in o-amido phenylsulfenic acids

The experimental observations discussed above clearly indicate that the cyclization of sulfenic acid **3** to sulfenyl amide **7** is a slow process and the *o*-amido thiophenols may not be suitable models to study the redox regulation that takes place at the active site of PTP1B. To find out the reason for the slow cyclization of the such sulfenic acids, we carried out density functional theory (DFT) calculations on sulfenic acids **3**, **4**, **20** and **21** (Schemes 2 and 8) using B3LYP/6-31G(d) level of theory [13]. We used hybrid B3LYP functional with 6-31G(d) basis set calculations as they have recently been used successfully by us [9] and Boyd and co-workers [15] to study the cyclization of sulfenic acids. Recently, Bayse and co-workers [16] have used solvent-assisted proton exchange (SAPE) to study the cyclization of such selenium system.

To predict the most stable conformation of *o*-amido sulfenic acids, a conformational search on sulfenic acid **20** was performed. It has been observed that the conformer of **20** with an intramolecular S···O interaction is at least 1.3 kcal mol^{-1} more stable than other conformers (Fig. S1, Supporting information). This geometri-



Scheme 8. Cyclization reaction of sulfenic acids **20** and **21** to sulfenyl amides **22** and **23**.

cal arrangement is further supported by the crystal structures of **8** [9] and **14**, which also contain S···O nonbonded interactions. DFT calculations indicated the presence of intramolecular S···O interactions in the sulfenic acids **3**, **4**, **20** and **21** (Fig. 3) due to the involvement of $n_O \rightarrow \sigma^*_{S-OH}$ orbital interactions and the S···O distances are found to be much shorter than the sum of the van der Waal's radii of sulfur and oxygen atoms (3.25 Å) (Table 1). The S···O nonbonded interaction between a divalent sulfur and an oxygen atom attracts considerable current interest [17] Tomoda and co-workers recently suggested that the S···O nonbonded interactions not only regulate enzymatic functions, but also may stabilize folded protein structures [18] The NBO analysis [11] at B3LYP/6-31G(d) level of theory on **3**, **4**, **20** and **21**, suggests that the stabilization energy due to $n_O \rightarrow \sigma^*_{S-OH}$ orbital interaction ($E_{S\cdots O}$) (NBO second order perturbation energy) is about 13 kcal mol^{-1} (Table 1). Further, the generation of a bond critical point (bcp) between the oxygen and sulfur atom and a ring critical point (rcp) for the resulting 5-membered ring clearly indicates the presence of S···O nonbonded interaction in **3**, **4**, **20** and **21** (Table 1). Due to these interactions, the –OH group adopts a position *trans* to the S···O interaction, leading to an almost linear arrangement of the O···S–O moiety (Table 1). Thus the cyclization partners –OH and –NH stay far away from each other and this may be the reason for the slow cyclization of such sulfenic acids to their corresponding sulfenyl amides. As S···O nonbonded interaction energies and electron densities at the bond critical point ($\rho_{S\cdots O}$) do not change much with the change in the substituent on the nitrogen atom, we assume that the cyclization efficiency will not depend on the substituent on the nitrogen atom.

2.4.2. Role of addition groups near the sulfenic acid (–SOH) moiety

We have previously shown that the sterically hindered thiol **9**, in which the 6-H in compound **2** has been replaced by an oxazoline moiety (Scheme 3), reacts with H_2O_2 to produce the sulfenyl amide **11** in a quantitative yield [9]. We proposed that the introduction of the oxazoline ring in the 6-position provides steric hindrance in the thiol **9** to prevent the oxidation of thiol to the corresponding disulfide **12** [9] and the S···N interactions [19] between the sulfur atom and the 5-membered heterocyclic nitrogen atom in the sulfenic acid may force the –OH moiety to approach the –NH– group facilitating the cyclization reaction to form expected sulfenyl amide. Further, theoretical studies suggested a neighboring group participation of the oxazoline ring in the transition state of **10** [9]. Herein, we studied the role of the additional substituent at the 6-position of the phenyl ring of sulfenic acids such as **10** on the cyclization reaction by using NBO and AIM analyses. Interestingly, because of the steric hindrence of the additional substituents at the 6-position of the phenyl ring, the *ortho*-amide oxygen atom of sulfenic acid **10** cannot involve in a noncovalent interaction and unlike sulfenic acids **3**, **4**, **20** and **21** the linear arrangement of O···S–OH is not possible in **10**. In contrast, the additional oxazoline ring in **10** forces the OH group towards the NH group. In addition to that, the oxazoline nitrogen atom forms a nonbonded S···N interaction (S···N = 2.83 Å; $E_{S\cdots N}$ = 2.95 kcal mol^{-1}; ρ_{bcp} = 0.019 au) due to $n_N \rightarrow \sigma^*_{S-OH}$ orbital interaction. DFT calculations and AIM analysis also suggested the presence of such stabilizing S···N interaction (S···N = 2.76 Å; $E_{S\cdots N}$ = 6.03 kcal mol^{-1}; ρ_{bcp} = 0.020 au) between the oxazoline nitrogen and the sulfur atom [$n_N \rightarrow \sigma^*_{S-N}$ orbital interaction] in the sulfenyl amide **11**. The crystal structure of **11** also confirms the presence of strong S···N interactions [9]. Similarly, in sulfenic **24** where the alcoholic moiety in **10** has been replaced by a hydrogen atom, a similar geometrical arrangement and S···N nonbonded interaction were observed by NBO and AIM analyses. Therefore, the oxazoline rings in **10** and **24** not only prevent the S···O interaction by steric hindrance to force the –OH moiety closer to the –NH– group and but also form additional nonbonded S···N interactions, which may facilitate the cyclization process.

2.4.3. Isosteric replacement and importance of steric effect

One way to evaluate the importance of steric and electronic effects in the cyclization reaction is the isosteric replacement of heteroatoms of the oxazoline ring in sulfenic acids **10** and **24**. The isosteric replacement of the nitrogen and oxygen atoms with –CH– and –CH_2– groups, respectively, are expected to retain the steric hindrance at the 6-postion of the phenyl ring, but would alter the electronic properties around the sulfenic acid moiety (–SOH). As expected, DFT calculations suggest that the introduction of the 5-membered ring having no heteroatoms does maintain the steric bulkiness of the 6-substituent in the sulfenic acids and this steric hindrance is sufficient to prevent any nonbonded S···O interactions between the sulfur and the carbonyl oxygen atom in

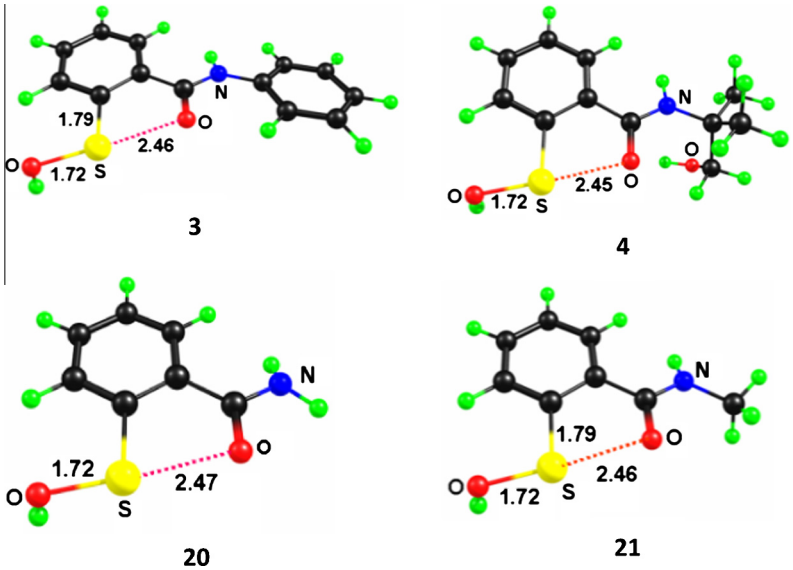


Fig. 3. B3LYP/6-31G(d) level optimized geometries and AIM structures of sulfenic acids **3**, **4**, **20** and **21.**

Table 1
Structural parameters and NBO second order perturbation energy and AIM parameters for the sulfenic acids **3**, **4**, **20** and **21** calculated at B3LYP/6-31G(d) level of theory.

Compd. (R group on N)	$r_{S\cdots O}$ (Å)	<O–S–O (°)	$E_{S\cdots O}$ (kcal mol^{-1})	$\rho_{S\cdots O}$ ea$_0^{-3}$	ρ_{rcp} ea$_0^{-3}$
3 (Ph)	2.457	176.6	14.00	0.0358	0.0202
4 [C(Me)$_2$CH$_2$OH]	2.448	176.8	14.75	0.0354	0.0200
20 (H)	2.471	176.7	13.69	0.0338	0.0195
21 (Me)	2.462	176.7	14.11	0.0336	0.0201

compounds **26** and **28** (see Supporting information for optimized structures). However, as the heteroatom-sulfur nonbonded interaction is absent in **26** and **28** their cyclization efficiency to form sulfenyl amides **27** and **29** is predicted to be lower than that of the conversion of sulfenic acids **10** and **24** to sulfenyl amides **11** and **25**. Further, when a *t*-butyl group was introduced at the 6-position of the *o*-amido phenylsulfenic acid (compound **30**, Fig. 4), similarly to sulfenic acids **26** and **28**, the optimized structure of **30** shows that the *t*-butyl group pushes the –OH group close to the –NH– group. Therefore, it can be concluded that additional substituent at the 6-position of *o*-amido sulfenic acids can facilitate the cyclization process by steric and electronic effects. The substituents that can contribute both steric and electronic effects simultaneously will be more effective in facilitating the cyclization process.

2.4.4. Effect of amide groups near the sulfenic acid moiety (-SOH)

In the PTP1B protein, the amide residues nearby the sulfenic acid moiety can modulate the electronic environments around the sulfur atom. Such an involvement of Gln residue in the antioxidant selenoenzyme glutathione peroxidase [20] has been proposed by Bayse and co-workers with the help of theoretical calculations [21]. In this regard, we have done theoretical calculations on sulfenic acid **32**, as a model, to mimic the role of amide carbonyl groups near the sulfenic acid moiety of PTP1B. Similar to the oxazoline ring discussed above, the S···O interactions between the sulfur atom and the amide carbonyl oxygen at the 6-position of the phenyl ring in the sulfenic acid **32** force the –OH moiety to approach the –NH– group. The S···O interaction in **32** [S···O = 2.53 Å; $E_{S\cdots O}$ = 9.72 kcal mol^{-1}] due to $n_O \rightarrow \sigma^*_{S-OH}$ orbital interation may contribute to the effective cyclization of **32**–**33**. DFT calculations also suggested the presence of S···O nonbonded interaction in the sulfenyl amide **33** [S···O = 2.57 Å; $E_{S\cdots O}$ = 9.21 - kcal mol^{-1}]. It is interesting that the nearby amide groups can push cyclization partners –OH and –NH– moieties in sulfenic acids close to each other by steric effect and additionally contribute to the effective cyclization *via* S···O nonbonded interactions. Therefore, it is likely that the amido groups near the sulfenic acid moiety may play important roles in the cyclization of the PTP1B sulfenic acid (PTP1B–SOH) to sulfenyl amides.

3. Conclusions

In summary, we have modelled the unusual transformation of the PTP1B sulfenic acid to the corresponding cyclic sulfenyl amide by using small molecule chemical models. It is found that the cyclization of an *o*-amido phenylsulfenic acids to sulfenyl amides is a slow process and the sulfenic acid produced *in situ* from H_2O_2-mediated oxidation of *o*-amido thiophenols can be trapped by using trapping agents such as CH_3I under suitable conditions. Theoretical studies suggest that the *o*-amido phenylsulfenic acids are stabilized by nonbonded S···O interactions between the lone pair of *ortho*-amido oxygen and antibonding orbital of S–OH bond ($n_O \rightarrow \sigma^*_{S-OH}$ orbital interactions). Due to this nonbonded interaction, the –OH group adopts a position *trans* to the S···O interaction, leading to an almost linear arrangement of the O···S–O moiety, which keeps the cyclization partners –OH and –NH groups far away from each other. This may be the reason for the slow cyclization of such sulfenic acids to their corresponding sulfenyl amides. As S···O nonbonded interaction energies and electron densities at the bond critical point ($\rho_{S\cdots O}$) do not change much with the change in the substituent on the nitrogen atom of *o*-amido phenylsulfenic acids, it can be concluded that the cyclization efficiency of these sulfenic acids to their corresponding sulfenyl amides will not depend on the substituent on the nitrogen atom.

DFT calculations further suggest that the introduction of a group that can induce steric effect can prevent the S···O interaction and force the –OH group close to the –NH– group, which may help in their cyclization to the sulfenyl amides. NBO analysis suggests that the introduction of groups such as an oxazoline ring

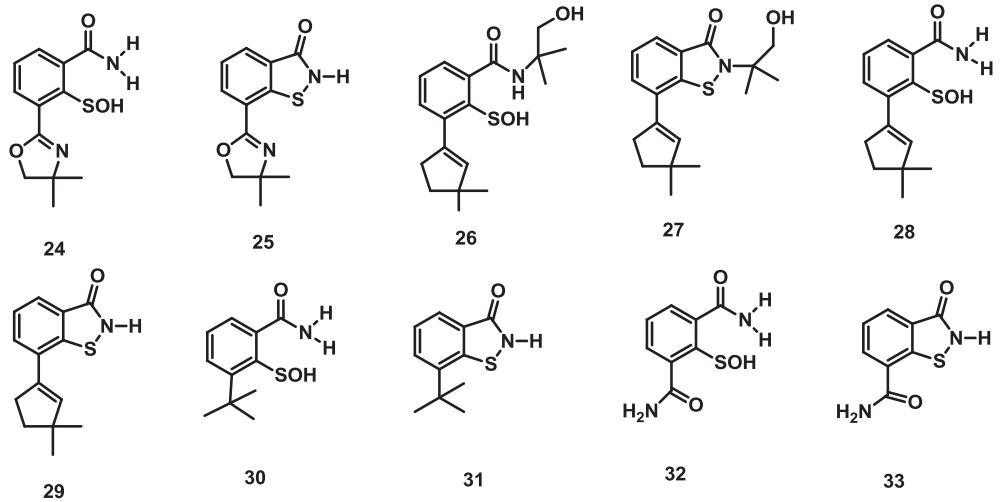


Fig. 4. Chemical structures of model sulfenic acids and sulfenyl amides.

that can induce steric effect as well as electronic effects due to the presence of the heteroatoms that can engage in nonbonded interation with the sulfenic acid moiety may be more effective in improving the cyclization efficiency to form sulfenyl amides. Further, it was observed that amide groups near the sulfenic acid moiety can induce both steric and electronic effetcs, which can help in efficient conversion of sulfenic acids to sulfenyl amides. This suggests that amido groups from amino acids other than the cyclization partners Cys215 and Ser216, that are close to the sulfenic acid moiety may play important roles in the cyclization of the PTP1B sulfenic acid (PTP1B–SOH) to sulfenyl amides. Therefore, it is important to consider both the steric and electronic environments around sulfenic acid moiety in PTP1B for designing synthetic models for the active site of the enzyme.

4. Experimental section

4.1. General procedure

All chemicals were of the highest purity available. All experiments were carried out under anaerobic conditions 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. Liquid state NMR spectra were recorded in $CDCl_3$ as a solvent. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Bruker Avance 400 NMR Spectrometer using the solvent as an internal standard for ^1H and ^{13}C. Chemical shifts (^1H, ^{13}C) are cited with respect to tetramethylsilane (TMS). Thin-layer chromatography analyses were carried out on pre-coated silica gel plates (Merck) and spots were visualized by UV irradiation. Column chromatography was performed on glass columns loaded with silica gel or on automated flash chromatography system (Biotage) by using preloaded silica cartridges.

4.1.1. Synthesis of 2-(2-(methoxycarbonyl)ethylthio)benzoic acid

To a stirred solution of thiosalicylic acid (1 g, 6.4 mmol) in dry THF (10 mL) under nitrogen was added triethylamine (1.3 g, 1.8 mL, 12.8 mmol) and methyl acrylate (671 mg, 702 mL, 7.8 mmol). The resulting solution was stirred at 25 °C under nitrogen for 24 h. The solution was then acidified with 10% H_2SO_4. The white precipitate was removed by filtration and the filtrate was extracted with diethyl ether (3 × 30 mL). The combined filtrates were dried over anhydrous Na_2SO_4 and evaporated to dryness to give a white solid, which was purified by flash chromatography to give a white powder (1.25 g, 83%). ^1H NMR ($CDCl_3$) δ 8.13 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.6 and 7.8 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.23 (t, J = 7.2 and 7.6 Hz, 1H), 3.73 (s, 3H), 3.25 (t, J = 7.2 and 7.6 Hz, 2H), 2.75 (t, J = 7.6 and 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.2, 171.0, 141.6, 133.2, 132.6, 126.6, 125.6, 124.4, 52.1, 33.0, 26.9. MS (TOF MS ES$^+$) m/z 263.0415 [M+Na]$^+$.

4.1.2. Synthesis of **14**

The thiol **1** (10 mg, 0.044 mmol) in acetonitrile (1.1 mL) was treated with sodium phosphate buffer (1.28 mL, 500 mM, pH 7.5) and methyl iodide (1.28 mL). To this mixture was added hydrogen peroxide (5.5 μL, 8.82 M in water) and the reaction was vigorously stirred (final concentrations: **1**, 11.9 mM; H_2O_2, 12.0 mM; buffer, 174.6 mM, pH 7.5; CH_3I, 5.58 M; acetonitrile, 30% by volume). The excess methyl iodide was removed by passing a stream of nitrogen over the mixture in a well-ventilated hood after 1.5 h. The reaction mixture was then extracted with ether twice and the organic layer was washed with water followed by brine. The organic layer was further dried over anhydrous sodium sulfate and the solvent was removed under vacuum. The crude reaction mixture was then purified by flash column chromatography to yield **14** white solid in ~10% yield. ^1H NMR (DMSO-d6) δ 10.60 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.86 (t, J = 8.0 and 8.0 Hz, 1H), 7.69–7.72 (m, 3H), 7.37 (t, J = 8.0 and 8.0 Hz, 2H), 7.14 (t, J = 8.0 and 8.0 Hz, 1H), 2.82 (s, 3H); ^{13}C NMR (DMSO-d6) δ 165.1, 148.7, 139.0, 132.7, 130.8, 129.2, 128.4, 124.7, 124.1, 120.9, 45.1; MS (TOF MS ES$^+$) m/z 282.0569 [M+Na]$^+$.

4.1.3. Synthesis of **15**

To a stirred solution of **7** (100 mg, 0.44 mmol) in CH_2Cl_2 (10 mL) under nitrogen was added thiophenol (46 μL, 0.45 mmol). The reaction mixture was stirred for 12 h at ambient temperature under nitrogen. The solvent was removed under reduced pressure to give a yellow oil which was the purified by flash column chromatography to give compound **15** in 80% yield (119 mg). ^1H NMR ($CDCl_3$) δ 7.88 (d, J = 8.0 Hz, 1H), 7.80 (s, 1H), 7.63 (t, J = 4.0 and 4.0 Hz, 3H), 7.49 (d, J = 8.0 Hz, 2H), 7.44 (t, J = 8.0 and 8.0 Hz, 1H), 7.38 (t, J = 8.0 and 8.0 Hz, 3H), 7.28–7.31 (m, 3H), 7.22 (d, J = 4.0 Hz, 1H), 7.17 (t, J = 8.0 and 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 165.7, 137.8, 137.5, 136.2, 134.4, 131.5, 129.1, 128.4, 127.6, 127.3, 126.7, 124.9, 120.2. MS (TOF MS ES$^+$) m/z 360.0461 [M+Na]$^+$.

4.1.4. Synthesis of **16**

To a stirred solution of **8** (100 mg, 0.45 mmol) in CH_2Cl_2 (10 mL) under nitrogen was added benzenethiol (46 μL, 0.45 mmol). The reaction mixture was stirred for 12 h at ambient temperature under nitrogen. The solvent was removed under reduced pressure to give a yellow oil which was the purified by flash column chromatography to give compound **16** in 70% yield (104 mg). ^1H NMR ($CDCl_3$) δ 7.77 (d, J = 8.0 Hz, 1H), 7.48 (d, 7.6 Hz, 3H), 7.38 (t, J = 7.6 and 8.0 Hz, 1H), 7.23–7.32 (m, 4H), 6.14 (s, 1H), 3.65 (s, 2H), 3.73 (s, 2H), 1.42 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 168.0, 136.2, 135.8, 134.6, 130.7, 128.7, 127.9, 127.3, 127.2, 126.9, 126.3, 69.8, 56.5, 24.2. MS (TOF MS ES$^+$) m/z 356.0738 [M+Na]$^+$.

4.1.5. Synthesis of **17**

To a stirred solution of **7** (100 mg, 0.45 mmol) in CH_2Cl_2 (10 mL) under nitrogen was added 4-methyl-thiophenol (56 mg, 0.45 mmol). The reaction mixture was stirred for 12 h at ambient temperature under nitrogen. The solvent was removed under reduced pressure to give a yellow oil which was the purified by flash column chromatography to give compound **17** in 75% yield (116 mg). ^1H NMR ($CDCl_3$) δ 7.89 (d, J = 8.0 Hz, 1H), 7.79 (s, 1H), 7.58–7.64 (m, 3H), 7.44 (t, J = 8.0 and 8.0 Hz, 1H), 7.37 (t, J = 8.0 and 8.0 Hz, 4H), 7.30 (t, J = 4.0 and 8.0 Hz, 1H), 7.16 (t, J = 8.0 and 4.0 Hz, 1H), 7.08 (d, J = 8.0 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.7, 137.8, 137.7, 137.6, 134.8, 132.8, 131.5, 130.0, 129.1, 129.0, 128.8, 127.9, 126.9, 124.8, 120.2, 21.1. MS (TOF MS ES$^+$) m/z 374.0762 [M+Na]$^+$.

4.1.6. Synthesis of **18**

To a stirred solution of 2-(2-(methoxycarbonyl)ethylthio)benzoic acid (264 mg, 1.1 mmol), DCC (336 mg, 1.64 mmol) and DMAP (26.8 mg, 20 mol%) in dry THF (12 mL) was added aniline (152 mL, 1.64 mmol) and the mixture allowed to stir at 25 °C for 12 h under nitrogen. The solvent was removed by rotary evaporation and the resulting oil taken up in diethyl ether and filtered to remove dicyclohexyl urea (DCU). The filtrate was then washed with 5% H_2SO_4, followed by water and saturated NaCl solution. The ether layer was then dried over anhydrous sodium sulfate, filtered, and evaporated to give a yellow oil, which was then purified by a flash column chromatography to give **18** as a white powder (205 mg, 65%). ^1H NMR ($CDCl_3$) δ 8.65 (s, 1H), 7.78 (d, J = 7.2 Hz, 1H), 7.69 (d,

J = 8.0 Hz, 2H), 7.51 (d, J = 7.6 Hz, 1H), 7.34–7.45 (m, 4H), 7.16 (t, J = 7.6 and 7.2 Hz, 1H), 3.58 (s, 3H), 3.19 (t, J = 6.8 and 7.2 Hz, 2H), 2.63 (t, J = 6.8 and 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.1, 165.9, 137.9, 132.4, 132.3, 131.0, 130.0, 129.6, 127.6, 124.6, 120.0, 52.0, 34.0, 33.0. MS (TOF MS ES$^+$) m/z 338.0820 [M+Na]$^+$.

4.1.7. Synthesis of **19**

To a stirred solution of 2-(2-(methoxycarbonyl)ethylthio)benzoic acid (382 mg, 1.59 mmol), DCC (485 mg, 2.38 mmol) and DMAP (38 mg, 20 mol%) in dry THF (20 mL) were added the 2-amino-2-methyl propan-1-ol (213 mg, 230 mL, 2.38 mmol) and the mixture allowed to stir at 25 °C for 24 h under nitrogen. The solvent was removed by rotary evaporation and the resulting oil taken up in diethyl ether and filtered to remove dicyclohexyl urea (DCU). The filtrate was then washed with 5% H_2SO_4, followed by water and saturated NaCl solution. The ether layer was then dried over anhydrous sodium sulfate, filtered, and evaporated to give a yellow oil, which was then purified by a flash column chromatography to give **19** as a white powder (270 mg, 60%). ^{1}H NMR ($CDCl_3$) δ 7.47 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 8.0 and 7.6 Hz, 1H), 7.22 (t, J = 7.2 and 8.0 Hz, 1H), 6.52 (s, 1H), 4.52 (s, brd, 1H), 3.65 (s, 2H), 3.56 (s, 3H), 3.10 (t, J = 7.2 and 6.8 Hz, 2H), 2.55 (t, J = 6.8 and 7.2 Hz, 2H), 1.34 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.1, 167.8, 137.7, 130.9, 129.5, 127.7, 126.4, 69.0, 55.9, 33.0, 28.9, 23.5. MS (TOF MS ES$^+$) m/z 334.1091 [M+Na]$^+$.

4.2. Computational methods

All calculations were performed using Gaussian98 or Gaussian03 suite of quantum chemical programs [22]. The hybrid Becke 3-Lee-Yang-Parr (B3LYP) exchange correlation functional was applied for DFT calculations [11]. Geometries were fully optimized at B3LYP level of theory using the 6-31G(d) basis sets. Orbital interactions were analyzed using the Natural Bond Orbital (NBO) method at the B3LYP/6-31G(d) level. The topology of the electron density was examined with the AIM2000 software package [23] using the wave functions obtained by the B3LYP/6-31G(d) computational methods.

4.3. X-ray crystallography

X-ray crystallographic studies were carried out on a Bruker CCD diffractometer with graphite-monochromatized Mo Kα radiation (l = 0.71073 Å) controlled by a Pentium-based PC running on the SMART software package [24]. 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 [25]. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were assigned idealized locations. Empirical absorption corrections were applied to all structures using SADABS [26,27]. The structure was solved by direct method (SIR-92) and refined by full-matrix least-squares procedure on F^2 for all reflections (SHELXL-97) [28].

4.3.1. Crystal data for **14**

$C_{14}H_{15}N_1O_3S$; M_r = 277.3, Monoclinic, space group P2(1)/c, a = 9.6353(10), b = 7.1392(7), c = 19.7728(20) Å, β = 98..918(2)°, V = 1343.70(5) Å^3, Z = 4, r_{calcd} = 1.37 g/cm, Mo Kα radiation (l = 0.71073 Å), T = 293(2) K; R_1 = 0.050, wR_2 = 0.110 ($I > 2\sigma(I)$); R_1 = 0.076, wR_2 = 0.124 (all data).

Acknowledgements

This study was supported by the Department of Science and Technology (DST), New Delhi, India. The author is grateful to Prof. G. Mugesh for his useful suggestions, encouragements and help in the preparation of the manuscript. The author also thanks the Supercomputer Engineering and Research Centre (SERC), Indian Institute of Science (IISc) for computing facilities.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2013.05.044.

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