New insights into the chemical behavior of S-oxide derivatives of thiocarbonyl-containing antitubercular drugs and the influence on their mechanisms of action and toxicity

Jean-Luc Stigliani, Vania Bernardes-Génisson

New insights into the chemical behavior of *S*-oxide derivatives of thiocarbonyl-containing antitubercular drugs and the influence on their mechanisms of action and toxicity

Nouvelles connaissances sur le comportement chimique des dérivés S-oxyde des médicaments antituberculeux contenant un groupe thiocarbonylé et l'influence sur leurs mécanismes d'action et de toxicité

J.L. Stigliani and V. Bernardes-Génisson*

CNRS, Laboratoire de Chimie de Coordination, LCC, UPR 8241, 205 Route de Narbonne, BP 44099, F-31077 Toulouse, Cedex 4, France.
Université de Toulouse, Université Paul Sabatier, UPS, 118 Route de Narbonne, F-31062, Toulouse, Cedex 9, France.

Summary

Objectives: This work aims at getting more insights into the distinct behavior of *S*-oxide derivatives of thiocarbonyl-containing antitubercular drugs, in order to better understand their mechanism of action and toxicity.

Methods: Computational calculation of relative free energy ($\Delta\Delta G$) of *S*-oxide tautomers (sulfine R-C(=S=O)NH_2), sulfenic acid (R-C(S-OH)=NH) and sulfoxide (R-C(SH=O)=NH)) derived from thioamide and thiourea antitubercular drugs and an update of the literature data with a new point of view about how the structural features of oxidized primary metabolites (*S*-oxide) can influence the outcome of the reactions and be determinant for the mechanisms of action and of toxicity of these drugs.

Results: The calculated free energy of *S*-oxide tautomers, derived from thioamide and thiourea-type antitubercular drugs, supported by some experimental results, revealed that *S*-oxide derivatives could be found under sulfine and sulfenic acid forms depending on their chemical structures. Thiocarbonyl compounds belonging to the thioamide series are firstly oxidized, in the presence of H_2O_2, into the corresponding *S*-oxide derivatives that are more stable under the sulfine tautomeric form. Otherwise, *S*-oxides of thiourea-type (acyclic and cyclic) compounds tend to adopt the sulfenic acid tautomeric form preferentially.

While the intermediate ethionamide-SO under sulfine form can be isolated and in the presence of H_2O_2 can undergo further oxidation by a mechanism yielding radical species that are toxic for *Mycobacterium tuberculosis* and human, thioacetazone-SO, found mainly into sulfenic acid form, is unstable and sufficiently reactive in biological conditions to intercept different biochemical pathways and manifests thus its toxicity.

Conclusion: Based on experimental and theoretical data, we propose that *S*-oxide derivatives of thioamide and thiourea-type antitubercular drugs have preference for distinct tautomeric forms. *S*-oxide of ethioamide is preferentially under sulfine form whereas *S*-oxide of thiourea compound as thioacetazone is mainly found under sulfenic acid form. These structural features lead to individual chemical reactivities that might explain the distinct mechanism of action and toxicity observed for the thioamide and thiourea antitubercular drugs.

KEYWORDS: thiocarbonyl compounds, sulfenic acid, sulfine, sulfinic acid, hydroxyl radical, *ab initio* calculations, flavine-containing monooxygenases; EthA; hepatotoxicity

Résumé

Objectifs: Ce travail vise à mieux comprendre le comportement distinct des dérivés *S*-oxyde des médicaments antituberculeux de type thiocarbonylé, afin d'obtenir plus d'informations sur leur mécanisme d'action et de toxicité.
Méthodes: Calcul théorique de l'énergie libre relative (ΔΔG) des tautomères *S*-oxyde (sulfine R-C(=S=O)NH_2), acide sulfénique (R-C(S-OH)=NH) and sulfoxyde (R-C(SH=O)=NH)) dérivés des médicaments antituberculeux de type thioamide et thiourée et une mise à jour des données de la littérature avec un nouveau point de vue sur la façon dont la structure des métabolites primaires oxydés (*S*-oxyde) peut influencer le devenir des réactions et être déterminant pour les mécanismes d'action et de toxicité de ces médicaments.
Résultats: Les énergies libres calculées pour les tautomères *S*-oxyde des médicaments antituberculeux de type thioamide et thiourée, supportées par quelques résultats expérimentaux, ont révélés que les dérivés *S*-oxyde pouvaient être préférentiellement trouvés sous l'une de deux formes tautomériques, sulfine or acide sulfénique, en fonction de leurs structures chimiques. Les composés thiocarbonylés appartenant à la série des thioamides ont été tout d'abord oxydés, en présence de H_2O_2, en dérivés *S*-oxyde correspondants qui seraient, d'après les calculs, plus stables sous la forme du tautomère sulfine. En contrepartie, les composés *S*-oxyde de type thiourée (acycliques et cycliques) tendraient à adopter préférentiellement la forme tautomérique de l'acide sulfénique.

Tandis que l'éthionamide-SO, sous forme de sulfine, est relativement stable et en présence de H_2O_2 subit une deuxième oxydation par un mécanisme générant des espèces radicalaires toxiques pour le *Mycobacterium tuberculosis* et l'homme, le thioacétazone-SO, sous forme d'acide sulfénique, est suffisamment réactif en milieu biologique pour intercepter différentes voies biochimiques et manifester ainsi sa toxicité. *Conclusion*: Sur la base des données expérimentales et théoriques, nous proposons que les dérivés *S*-oxyde des antituberculeux de type thioamide et thiourée ont une préférence pour des formes tautomères distinctes. Le *S*-oxyde du médicament antituberculeux éthioamide est préférentiellement sous la forme de sulfine tandis que le *S*-oxyde de la thioacétazone (thiourée) se trouve principalement sous forme d'acide sulfénique. Ces caractéristiques structurales conduisent à des réactivités chimiques propres qui pourraient donc expliquer les différents mécanismes d'action et de toxicité observés pour les médicaments antituberculeux contenant les fonctions thioamide et thiourée.

MOTS CLES: composés thiocarbonylés; acide sulfénique; sulfine, acide sulfinique, radical hydroxyle, calculs *ab initio*, monooxygenases à flavine; EthA; hépatotoxicité.

Introduction:

Tuberculosis (TB), whose causative agent is *Mycobacterium tuberculosis* (*Mtb*), remains one of the most important infectious diseases in the world causing over 1.3 million deaths annually and being responsible for latent (non-replicating) infections in more than 33% of population.[1] The first-line tuberculosis chemotherapy is based on the use of four drugs: rifampicin, isoniazid, ethambutol and pyrazinamide however, lately, this treatment has been become inefficient towards patients with infections caused by multidrug and extensively drug-resistant *Mtb* strains. In this context, the second-line antitubercular drugs have been occupying a more important place in the therapy of this disease. Among these drugs, some ones, embedding a thiocarbonyl group, have though limited clinical uses due to their severe hepatotoxicity effect. Indeed, the hepatotoxicity of biologically active thiocarbonyl-containing compounds was initially reported for thioacetamide (TA) (a carcinogen and teratogen compound used as fungicide, Fig. 1) by Fitzhugh OG *et al* and later by other authors.[2]

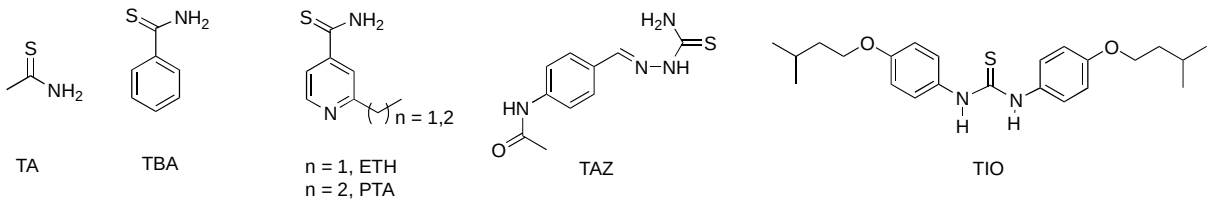


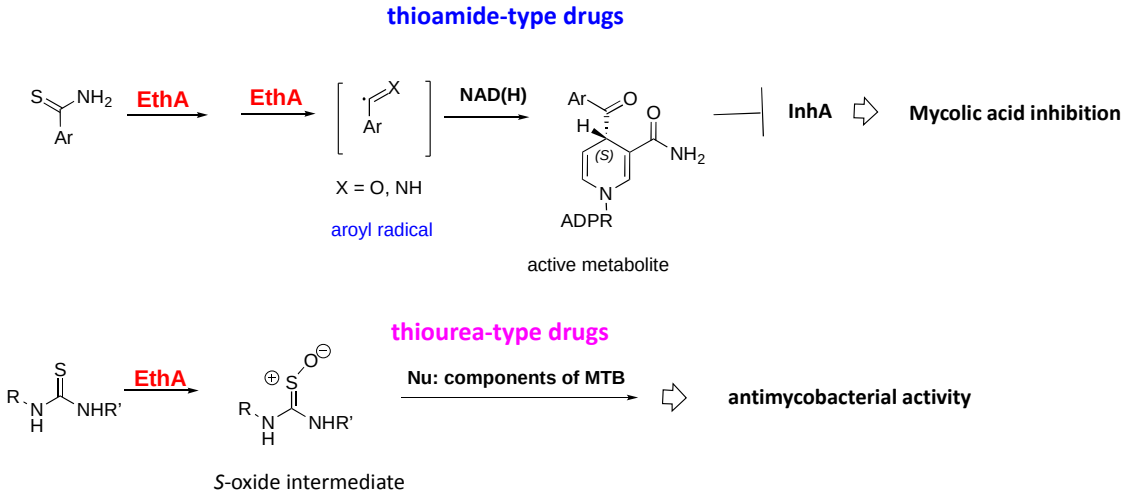
Figure 1: Some bioactive thiocarbonyl-containing compounds

Figure 1: Composés bioactifs contenant des groupes thiocarbonylés.

It was described that this compound, even using single doses, is responsible for serious liver injury and its prolonged administration in male rats can causes liver cancer. Later on, the toxicity of other thiocarbonyl compounds, including some with antitubercular activities as thiobenzamide (TBA), ethionamide (ETH), protionamide (PTA), thioacetazone (TAZ) and tiocarlide (TIO), were also reported[3] (Fig. 1).

The thiocarbonyl-containing antitubercular compounds, cited above, belong to two different chemical series: thioamide and thiourea/thiocarbamide. These medicines are in fact pro-drugs of bioprecursor-type. It means that they must undergo a chemical transformation, in these cases an enzymatic oxidative reaction, to generate the active metabolic species. Despite their distinct chemical functionalities and structures, they are activated inside *Mtb* by a same flavine-containing monooxygenase enzyme (EthA).[3e,4] However, it is reported in the literature that both series do not have the same kind of active metabolite and not aim at the same target. In the case of ETH and PTA (thioamide series), it was evidenced that, during their activation, 3-alkyl isonicotinoyl radicals are formed after two oxidation steps and they are indeed the reactive species that react with the cofactor NAD(H) to generate the ultimate active metabolite, which is inhibitor of InhA enzyme and toxic for *Mtb* (scheme 1).[3e,5] Otherwise, for thiourea-type antitubercular compounds, such as TAZ and TIO, there is much more limited information concerning their biological action. It is believed that these drugs, unlikely thioamide drugs, are active shortly after the first oxidation step by forming an *S*-oxide intermediate, which is reactive

towards nucleophilic (Nu:) components of *Mtb* such as mycothiol compound or cysteine residues of an essential enzyme of *Mtb* (scheme 1).[3e,6]



Scheme 1

Additionally, it is well agreed that similarly to the drug activation mechanisms that occur inside *Mtb*, such compounds (thioamides and thioureas) could also be bio-transformed in liver by human flavine-containing monooxygenase enzymes (FMOs), *via* a pathway known as oxidative desulfurization, to lead to harmful metabolites, which would be responsible for the observed hepatotoxicity of these drugs.[3e,7]

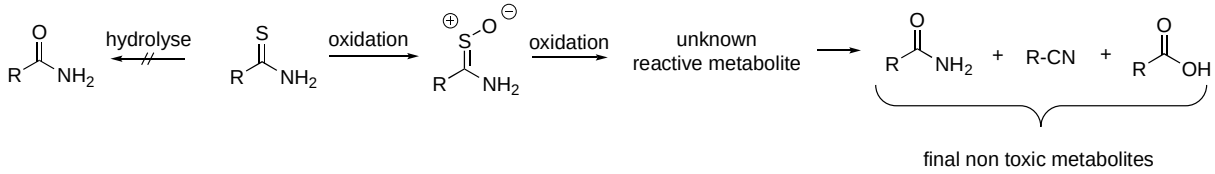
Despite numerous works carried out so far to get insights into the understanding of the mechanism of toxicity induced by thiocarbonyl compounds, the molecular basis of the oxidative desulfurization pathway and the identification of resulting toxic species are not yet elucidated and many questions remain without responses.

In this paper, we propose an update of (bio)-oxidation of thiocarbonyl-containing compounds supplemented with some computational calculations in an attempt to explain how the structural features of primary oxidative metabolites (*S*-oxide) might influence the outcome of the reactions and to be determinant for the mechanisms of action and toxicity of these drugs.

Results and discussion:

Thioamide derivatives

Thioacetamide and thiobenzamide are both models for hepatotoxicity and were thus widely investigated for the understanding of the toxicity of thiocarbonyl-containing compounds as ethionamide. Several authors have concluded that, like for thioamide-type antitubercular drugs activation (inside *Mtb*), two metabolic steps are required to produce toxic species[3b,8] and the metabolic oxidation is essentially carried out by FMOs that are enzymes found in the liver of mammals equivalent to EthA of *Mtb*. However, some authors have also described thioamide oxidation by cytochrome P-450 systems.[2c,9] The authors are also in agreement with the fact that thiocarbonyl groups are first oxidized into *S*-oxide (SO) derivatives. This first metabolite was, indeed by some times, isolated, identified and characterized as a transient intermediate.[3b] Furthermore, it is known that a slower second oxidation reaction occurs via a mechanism quite different from the first one. This step leads to the formation of a second oxidized intermediate, which was never isolated nor characterized, along with the corresponding amide, acid and nitrile derivatives (scheme 2). These final metabolites are non-toxic and do not produce liver injury as the sulfur parent compounds. The qualitative and quantitative composition of the latter non-toxic metabolites can vary in function of the substrate chemical nature as well as the studied oxidative conditions (medium pH).[3b] There are sufficient experimental evidences to affirm that the corresponding amide derivative is formed via an oxidative desulfurization pathway from the thiocarbonyl parent compound and not via a direct mechanism involving hydrolysis of thioamide group[7] (scheme 2). The fact the corresponding final metabolites (amide, acid and nitrile derivatives) do not exhibit the toxicity observed for the sulfur parent compound suggests that the reactive *S*-oxygenated intermediates, formed during the oxidative pathway, are in fact the actual toxic species.

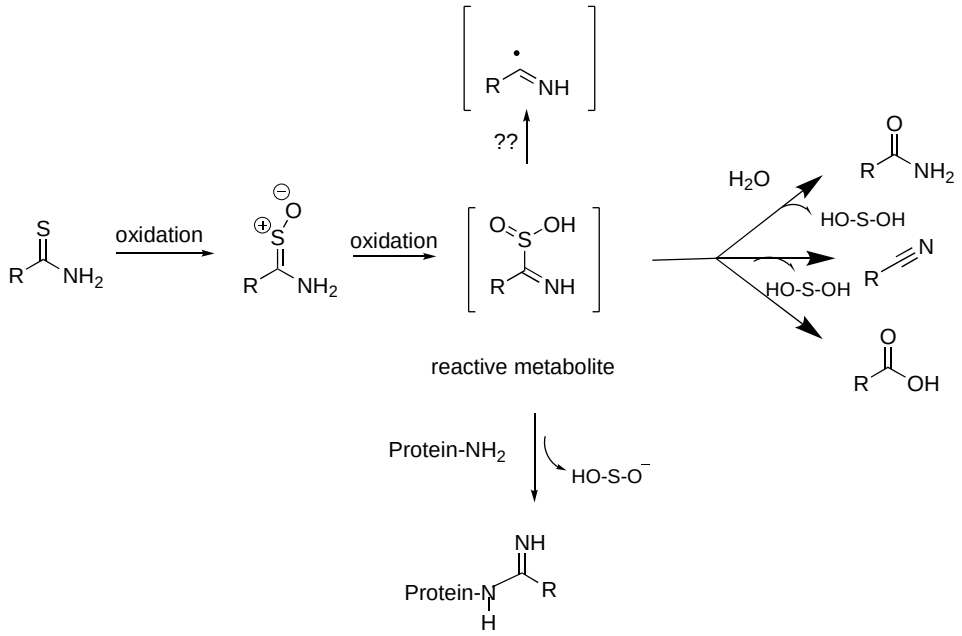


Scheme 2

Labeled experiments for ETH using H_2O_2 as oxidant agent and $H_2{}^{18}O$ as solvent, showed that a mixture of labeled (60%) and non-labeled amide (40%) was formed, suggesting thus that likely more than one reaction mechanism can be involved in the oxidative desulfurization process (scheme 3).[10] Furthermore, some experiments have shown that elimination of sulfur atom occurs at higher oxidation state (sulfur dioxide/sulfite)[10b,11] implicating thus oxidation of sulfur before its loss. Nevertheless, loss of sulfates was not detected when thionicotinamide was oxidized by peracetic acid.[10b,12]

To explain part of these results and action/toxicity mechanisms of the thiocarbonyl-containing compounds, as thioamides, it has been hypothesized for many years that *S*-oxide derivative, arisen from the first oxidation step, is relatively stable to undergo further oxidation and be converted into the putative corresponding sulfinic acid (ETH-SO_2H) (scheme 3). The latter is thus considered as the precursor of all non-toxic metabolites (amide, acid and nitrile) and also of aroyl radicals that in the case of ETH and PTA are the toxic species for *Mtb* (scheme 3). The mechanism of amide metabolite formation from the oxidation of the corresponding thioamide compound has more commonly attempted to be explained via a hydrolysis reaction upon the α carbon of the sulfinic acid compound (R-$C_\alpha(SO_2H)$=NH) (supposed to be formed after the second oxidation) followed by departure of the unstable $HOSO^-$ as good leaving group (scheme 3).[3b,3c] In addition, it was postulated that the corresponding sulfinic acid (R-$C_\alpha(SO_2H)$=NH) of ETH, although never isolated nor characterized, manifests the toxicity by acting as an acylating agent and hence establishing covalent binding to cellular nucleophiles as amine group of proteins (nucleophilic attack upon α-carbon of sulfinic acid function)[13a] (scheme

3). It is believed that resulting adducts are responsible for the lack of cell functionality and consequently for the well-known hepatotoxicity.[2c,3b]



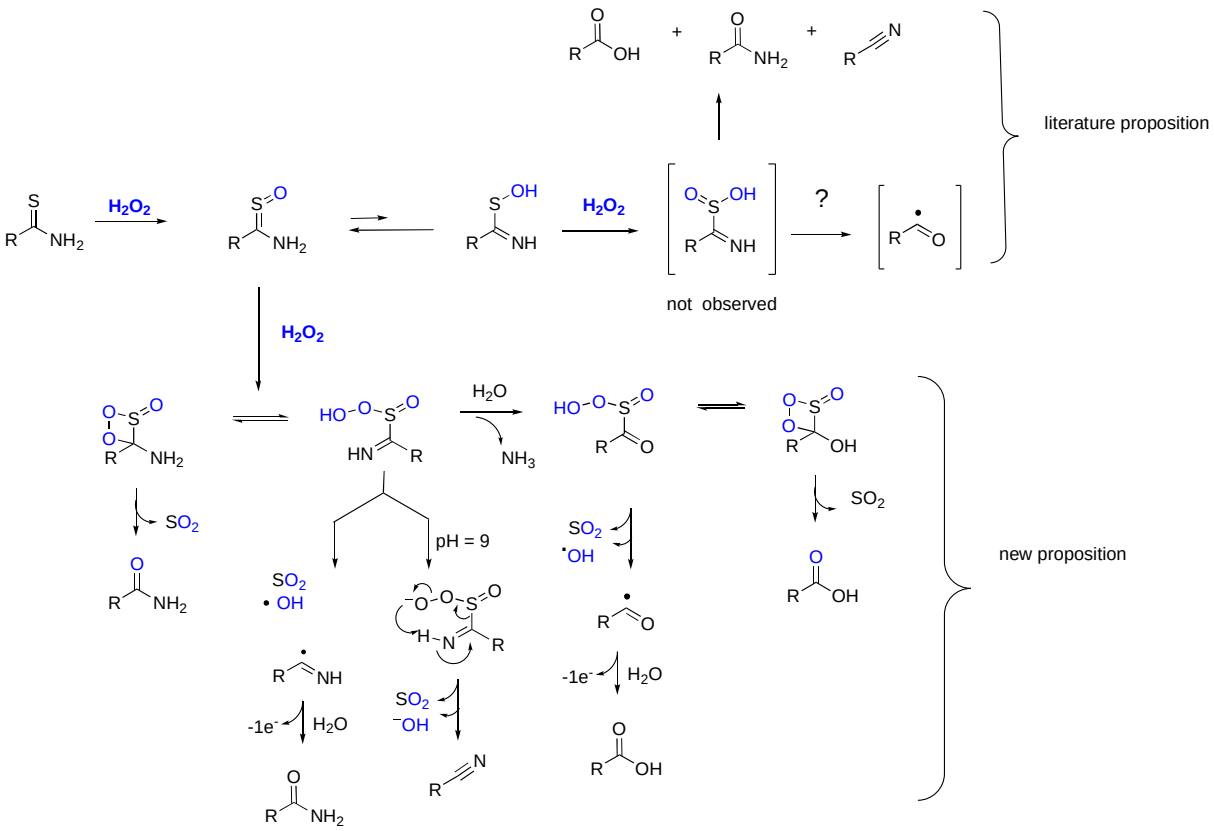
Scheme 3

In our opinion, these proposals suffer from several weaknesses. Firstly, the intermediate ($R\text{-}SO_2H$) was never identified/isolated; secondly the proposal justifying amide formation via a hydrolysis mechanism,[3a,3b,13a] as shown in scheme 3, is not sufficient to explain alone the results of labeled experiments (mentioned above), in which the oxygen of amide is not uniquely provided by water. Finally, no mechanistic proposition exists to explain how the isonicotinoyl radicals can be generated from sulfinic acid precursor during the ETH/PTA activation process, since homolytic cleavage of the C_{sp2}-S bond is not well documented in the literature (scheme 3).

Our recent studies on the oxidation of the antitubercular drug ETH, using a biomimetic oxidant system (H_2O_2), have also evidenced that amide, nitrile and acid metabolites are generated as final non-toxic compounds in the course of the second oxidation step.[10a] In addition, using this oxidant (H_2O_2), we have also demonstrated that radical species, which were assigned as isonicotinoyl and hydroxyl radical (EPR detection), are also concomitantly formed. It is worth to note that these data well correlated with those resulted from the enzymatic oxidation of ETH by EthA within *Mtb*, in which the isonicotinoyl radical is

generated and responsible by the formation of the ETH active metabolite (scheme 1). This allowed us to validate H_2O_2 as a biomimetic oxidant agent. Once again, in these conditions, no ETH-sulfinic acid (ETH-SO_2H) formation could be proven. This observation led us to query about the real role of the sulfinic acid derivative (R-SO_2H) and more precisely its function as an acylating agent and as precursor of the radical species and of non-toxic metabolites.

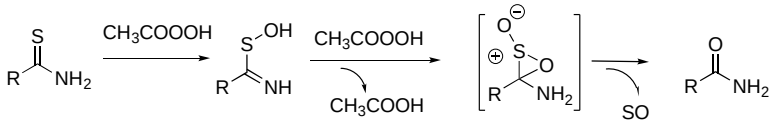
Taking into account all results presented above, we have suggested[10a] that the first metabolite (*S*-oxide) might preferentially exist, in the case of thioamide compounds, under sulfine (ETHS=O) tautomer form (scheme 4). This compound undergoes a supplementary reaction with H_2O_2 (via nucleophile attack of H_2O_2 upon sulfur atom followed by a cyclisation step or *via* a formal [2+2] cycloaddition reaction) leading in both cases to a same dioxathietane intermediate that can exist in equilibrium between opened and closed forms (scheme 4). These tautomeric structures can break down (from an homolytic cleavage of O-O bond) into isonicotinoyl radical and non-toxic metabolites by a pathway not involving sulfinic acid (R-SO_2H) as intermediate (scheme 4).



• in this mechanistic proposition bleu oxygens are provided by H_2O_2 and black oxygen are provided by water

Scheme 4 (adapted from ref.10a)

Recently and independently, H. Simoyi and collaborators,[10b] have also concluded that sulfinic acid derivative (R-SO_2H) might not be an intermediate in the ETH activation and the cleavage of the carbon-sulfur bond (desulfurization step) might occur upon unstable intermediate (scheme 5).



Scheme 5

This mechanism (extrusion of sulfur monoxide from *S*-oxathiirane *S*-oxide intermediate), if occurs, can explain the presence of non-labeled amide metabolites, formed when $H_2{}^{18}O$ was used in the experiments, however, the same does not serve to explain the labeled amide (when $H_2{}^{18}O$ is used) nor the formation of the isonicotinoyl radical that is the veritable reactive species involved in the anti-*Mtb* activity of ETH (Scheme 1).

In the light of the mechanism that we have proposed, that contrasts to others presented in the literature, the formation of amide (with oxygen providing from water or oxidant), nitrile and acid derivatives and also that of radical species (isonicotinoyl radical and hydroxyl radical) could be readily explained (scheme 4).

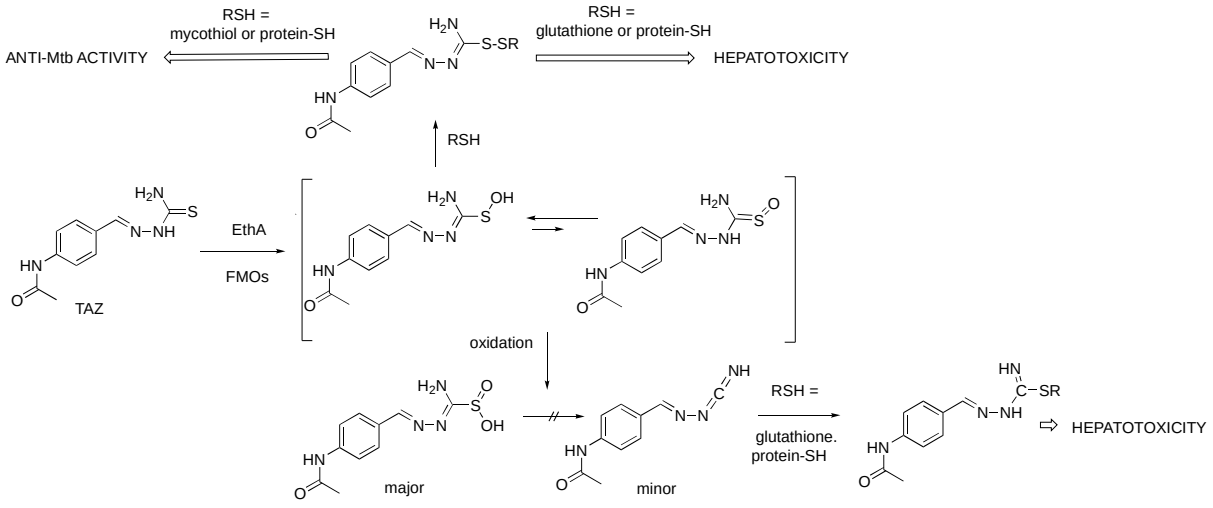
By analogy with ETH activation mechanism inside *Mtb*, it can be supposed that hepatic FMOs can similarly oxidize thioamide compounds into aroyl and hydroxyl radicals. Therefore, the hepatoxicity observed for thioamide compounds can be rationalized *via* the formation of isonicotinoyl radical that may function as an acylating agent, supporting thus the identification of drug-protein adducts reported by Hanzlik and co-workers[13b] and especially *via* the occurrence of the hydroxyl radical that is well accepted to be highly reactive and dangerous for health.[14]

Thiourea derivatives: thioacetazone and tiocarlide

With respect to thiourea-containing antitubercular drugs as TAZ and TIO, information about their chemical and biochemical oxidation mechanism, their mode of action, their toxicity and the chemical identity of the oxidized species are yet elusive. However, a number of interesting studies about the toxicity and identification of oxidative metabolites provided from other thiourea(thione)-containing xenobiotic compounds have been reported.[11,15] It was demonstrated that compounds embedding a thiourea moiety, similarly to thioamide-containing compounds, are excellent substrates for FMO-dependent *S*-oxygenation.[7b,11,16] Again, the oxidation of such compounds by monooxygenases is also believed to be associated to the observed hepatotoxicity. It is proposed, for this class of compounds, that the first oxidation of the organosulfur group results in formation of a sufficient reactive *S*-oxygenated intermediate that can intercept different biochemical pathways, manifesting thus its toxicity.

In 2006, R. Ortiz Montellano et co-workers[3d] have reported that *in vitro* EthA of *Mtb* initially oxidizes TAZ into the corresponding *S*-oxide intermediate, which is also considered

as the active metabolite responsible for the antibacterial activity of thiourea drugs, even though it has never been isolated. The authors also showed that this *S*-oxide intermediate could also undergoes, in a very lesser extent, supplementary oxidation yielding to sulfinic acid (TAZ-SO_2H) (major) and carbodiimide (minor) metabolites (scheme 6), which were unambiguously identified by SM and ^1H NMR analyses. These results are very surprising because this sulfinic acid (TZA-SO_2H) derivative, which could be synthesized, isolated and characterized, is not so unstable as advanced for ETH-SO_2H to justify its existence since never detected. Furthermore, it worth to underline that despite formation of TAZ-SO_2H metabolite, no antitubercular/toxicity action was assigned to its presence. Moreover, the authors have demonstrated that both final metabolites (sulfinic acid and carbodiimide) were derived from a supplementary oxidation of the corresponding initial *S*-oxide intermediate but TAZ-SO_2H was not the precursor of TAZ-carbodiimide (scheme 6) (via elimination of sulfoxylate ion, $HOSO^-$) as could be thought based on some mechanistic propositions.[3b] On the other hand, it is believed that *S*-oxide thiocarbonyl compounds (R-SO) as TAZ-SO and TIO-SO are indeed quite reactive towards sulfhydryl groups as glutathione and an eventual reaction with mycothiol (antioxidant molecule) of *Mtb* would be therefore responsible by the production of an oxidative stress inside of the bacteria[3e,7b] (scheme 6). This reaction could also take place with the TAZ-carbodiimide derivative (equivalent of nitrile compound in thioamide series) (scheme 6).

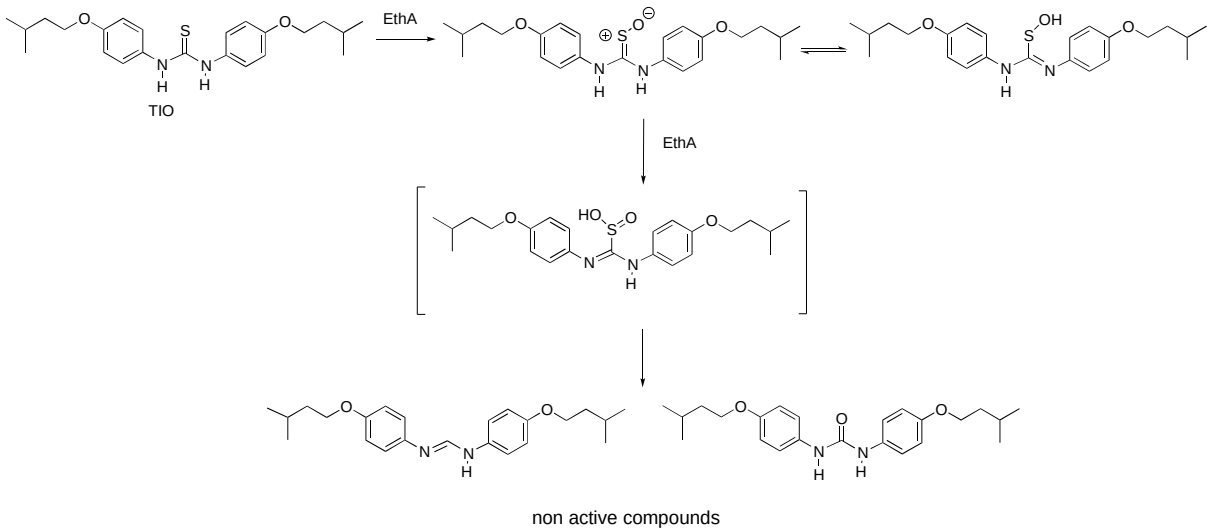


Scheme 6

In 2015, M. Jackson and coll.[6] have reported that TAZ and TIO could exert their antibacterial action inside *Mtb*, by a mechanism different from that based on the decreasing of the mycothiol concentration. They described that, after the first oxidation step, corresponding *S*-oxide derivatives of TIO and TAZ behave as active metabolite establishing a disulfide covalent bond with a cysteine residue (Cys61) of the HadA subunit of the dehydratase (belonging to *Mtb*-Fatty acid synthase II), and thus inhibiting the activity of this enzyme and hence the mycolic acid biosynthesis. *In vitro*, the activation of TIO by purified recombinant EthA from *M. smegmatis*, has shown by LC/MS analyses that, beyond unreacted TIO, the corresponding urea derivative, the formamidine compound and in a lesser extent two other compounds revealing a m/z = 417 compatible with TIO-SO (M = 416) were formed (Scheme 7). The authors have also proposed that formation of the corresponding urea and formamidine metabolites occurs, as proposed for ETH, through a desulfurization step from the sulfinic acid ($R\text{-}SO_2H$) intermediate, even though this latter has not been identified in the case of TIO.

By extrapolation to what occurs inside *Mtb*, it has been supposing that the toxicity of thiourea compounds can be manifested by reaction of electrophilic S-oxide intermediates with sulfhydryl groups either of glutathione, trigging thus an oxidative stress, or of protein cysteine residues modifying thus the protein structure and hence cell functionality.

Although different mechanisms can exist to explain the toxicity of these two compounds, altogether converge to the fact that reactive and/or toxic metabolites arise from the first oxidation (R-SO) and no antitubercular/toxicity action was assigned to sulfinic acid compound.



Scheme 7

The results presented above for thioamide and thiourea compounds indicated that the corresponding *S*-oxide derivatives, resulted from a first oxidation of thiocarbonyl compounds, have distinct reactivity and seem to have a major determinant role in the outcome of their mechanisms of action and toxicity and in the formation of final metabolites.

In attempt to explain these differences of behavior, we postulated that *S*-oxide type compound would exist in equilibrium between two different tautomers: sulfine (R-C(=S=O)NH_2) and sulfenic acid (R-C(S-OH)=NH) and their reactivity might be different and decisive for the outcome of reactions. While sulfenic acid (R-C(-S-OH)=NH) preferentially reacts as an electrophile, for example with sulfhydryl residues, the sulfine (R-C(-NH_2)=S=O) tautomer reacts with H_2O_2 leading to an unstable dioxathietane intermediate, that in turn can exist between a close and an open tautomeric forms. By this latter mechanism, *via* a homolytic cleavage of the peroxide bond, it would be possible to explain the generation of radical species as well as that of all final metabolites as amide/urea, acid, and nitrile/diimide derivatives (scheme 4).

Free Energy Calculation

To test this hypothesis, we performed a series of theoretical calculations, based on the determination of Gibbs free energy (ΔG) of different tautomeric forms of *S*-oxide derivatives of the six different compounds, enclosed on table 1. The cyclic thiourea compound (benzimidazoline-2-thione, BIT) was included in this theoretical study to complete the series of three thiourea members and three thioamide components. The tautomeric forms of organic compounds distinguish by the position of hydrogen atom attachment. In the case of *S*-oxide derivatives, the hydrogen can be attached to the oxygen (sulfenic acid), to the nitrogen (sulfine) or still to the sulfur (sulfoxide).

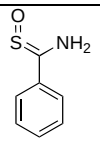
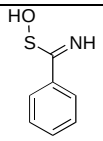
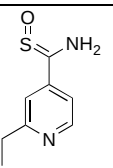
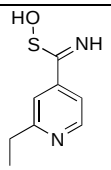
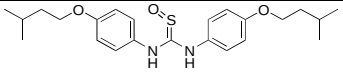
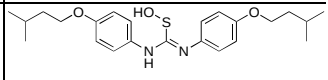
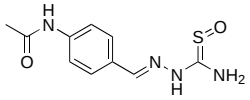
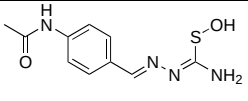
Sulfine/sulfenic acid tautomeric interconversions, related to the six compounds described on table 1, have been thus investigated by using DFT calculations. The results showed that the energetic stability order for the selected tautomers depends on the chemical series. For ETH, TA and TBA (thioamide series), sulfine (R-C(=S=O)NH_2) structure is more stable than the corresponding tautomer sulfenic acid (R-C(S-OH)=NH) (table 1) that in turn is much more stable than sulfoxide tautomer (R-C(SH=O)=NR') ($\Delta\Delta G$ = 22.37, 21.54, 21.35 kcal/mol for TA-SH=O, TBA-SH=O and ETH-SH=O, respectively). In fact, sulfoxide (R-C(SH=O)=NR') tautomers are very high in energy and are not relevant structures. In the case of ETH, the two other tautomers (sulfine/sulfenic acid) have an energy gap of about 3.5 kcal/mol and since tautomerisation of sulfine into sulfenic acid form is an endothermic process, the equilibrium is shifted toward sulfine compound. This result agrees with the X-ray data,[10a] for which the monooxygenated ethionamide compound (ETH-SO), in solid state, exists uniquely under sulfine form (R-C(=S=O)-NHR'). In contrast, in the thiourea series, the tendency is that sulfenic acid tautomer (R-C(S-OH)=NR') becomes as well as or even more stable than sulfines. This behavior is very clear for TAZ and the cyclic thiourea compound BIT, for which the difference of energy between sulfine and sulfenic acid tautomers can achieve 5.7 kcal/mol. The presence of an intramolecular hydrogen bonding (between N_{sp3} and HO) and the apparition of a conjugated system for TAZ and an aromatic

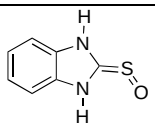
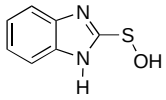
bicycle for BIT seem to be essential features allowing stabilization of sulfenic acid tautomers.

The above results are in accordance with the mechanism proposed for ETH by us, in which the reactivity of the *S*-oxide intermediate is based on the sulfine form that is indeed the major tautomer. For thiourea compounds, the mechanisms of action and toxicity proposed in the literature have been focused on the electrophilicity of SO compounds under sulfenic acid form, which has tendency to be, according the calculation, the favored structure.

Table 1: Computed relative free energies of the studied compounds into two tautomer forms

Table 1: Energies libres relatives calculées pour les composés étudiés dans deux formes tautomères

Parent molecule	Sulfine	ΔΔ kcal/mol	ΔΔG kcal/mol	Sulfenic acid
TA		0	3.17	
TBA		0	2.77	
ETH		0	3.50	
TIO		0	0.21	
TAZ		4.2	0.0	

BIT		5.77	0.0	

Experimental

Procedures

All the calculations were carried out with the GAUSSIAN 09 suite[17a]. Geometry optimizations were performed with the M06-2x[17b] density functional and the 6-311++G(2d,2p) basis set. The effect of solvation was described with the self-consistent reaction field (SCRF) method using the integral equation formalism polarizable continuum Model (IEFPCM) with water as solvent[17c]. Vibrational frequency calculations were performed to confirm the convergence to local minima and to calculate the unscaled zero-point-energy (ZPE) and the Gibbs free energy at 298 K.

Conclusion

Thionamides and thioureas are both oxidized at sulfur atom by H_2O_2 to their superior oxidative state, S-oxide intermediate. Experimental and theoretical investigations discussed in this work have provided new insights into the likely existence, in solution, of equilibrium of two oxidized tautomers, (sulfine and sulfenic acid) and the close relationship between their reactivity and their mechanism of action/toxicity. In the case of thioamide-containing compounds, sulfine form is the major one and leads, in oxidation conditions, to potential hepatotoxic radical species formation, while the studied thiourea-containing compounds have more tendencies to be found under sulfenic acid form that is very reactive towards cell nucleophile components.

In the light of these results, the consideration of the existence of these two tautomeric forms and their possible different chemical reactivity are elements that should be taken into account in drug design/development and in the study of potential hepatotoxic risk of thiocarbonyl-antitubercular drugs.

References

[1] WHO. Global tuberculosis report 2016; http://www.who.int/gho/tb/epidemic/cases_deaths/en/

[2] a) Fitzhugh OG, Nelson AA. Liver tumors in rats fed thiourea and thioacetamide. *Science* **1948**, *108*, 626-628. b) Hunter AL, Holscher MA, Neal RA. Thioacetamide induced hepatic necrosis. I. Involvement of the mixed-function oxidase enzyme system. *J. Pharmacol. Exp. Ther.* **1977**, *200*, 439-448. c) Chieli E, Malvaldi G. Role of the microsomal FAD-containing monooxygenase in the liver toxicity of thioacetamide *S*-oxide. *Toxicology* **1984**, *31*, 41-52. d) Chilakapati J, Korrapati MC, Hill RA, Warbritton A, Latendresse JR, Mehendale HM. Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide. *Toxicology* **2007**, *230*, 105-116.

[3] a) Newton RW. Side effects of drugs used to treat tuberculosis. *Scott Med. J.* **1975**, *20*, 47-49. b) Cashman JR, Hanzlik RP. Oxidation and other reactions of thiobenzamide derivatives of relevance to their hepatotoxicity. *J. Org. Chem.* **1982**, *47*, 4645-4650. c) Hillhouse JH, Blair IA, Field L. Thiono compounds. 7. Oxidation of thioamides in relation to adverse biological effects. *Phosphorus, Sulfur and Silicon and the Relat. Elem.* **1986**, *26*, 169-184. d) Qian L, Ortiz de Montellano PR. Oxidative activation of thioacetazone by the *Mycobacterium tuberculosis* flavin monooxygenase EtaA and human FMO1 and FMO3. *Chem. Res. Toxicol.* **2006**, *19*, 443-449. e) Nishida CR, Ortiz de Montellano PR. Bioactivation of antituberculosis thioamide and thiourea prodrugs by bacterial and mammalian flavin monooxygenases. *Chem. Biol. Interact.* **2011**, *192*, 21-25.

[4] a) Dover LG, Alahari A, Gratraud P, Gomes JM, Bhowruth V, Reynolds RC et al. EthA, a common activator of thiocarbamide-containing drugs acting on different mycobacterial targets. *Antimicrob. Agents Chemother.* **2007**, *51*, 1055-1063. b) Grzegorzewicz AE, Korduláková J, Jones V, Born SEM, Belardinelli JM, Vaquié A et al. A common mechanism of inhibition of the *Mycobacterium tuberculosis* mycolic acid biosynthetic pathway by isoxyl and thiacetazone. *J. Biol. Chem.* **2012**, *287*, 38434-38441.

[5] a) DeBarber AE, Mdluli K, Bosman M, Bekker L-G, Barry 3rd CE. Ethionamide activation and sensitivity in multi-drug resistant *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9677-9682. b) Wang F, Langley R, Gulten G, Dover LG, Besra GS, Jacobs WR

Jr et al. Mechanism of thionamide drug action against tuberculosis and leprosy. *J. Exp. Med.* **2007**, *204*, 73-78.

[6] Grzegorzewicz AE, Eynard N, Quémard A, North EJ, Margolis A, Lindenberger JJ et al. Covalent modification of the *Mycobacterium tuberculosis* FAS-II dehydratase by isoxyl and thioacetazone. *ACS Infect. Dis.* **2015**, *1*, 91-97.

[7] a) Dodge AG, Richman JE, Johnson G, Wackett LP. Metabolism of thioamides by *Ralstonia pickettii* TA. *Appl. Enviromental Microbiology* **2006**, *72*, 7468-7476. b) Henderson MC, Siddens LK, Krueger SK, Stevens JF, Kedzie K, Fang WK et al. Flavin-containing monooxygenase *S*-oxygenation of a series of thioureas and thiones. *Toxicol. Appl. Pharmacol.* **2014**, *278*, 91-99.

[8] Henderson MC, Siddens LK, Morré JT, Krueger SK, Williams DE. Metabolism of the anti-tuberculosis drug ethionamide by mouse and human FMO1, FMO2, FMO3 and mouse and human lung micrososmes. *Toxicol. Appl. Pharmacol.* **2008**, *233*, 420-427.

[9] Tynes RE, Hodgson E. Oxidation of thiobenzamide by the FAD-containing and cytochrome P-450-dependent monooxygenases of liver and lung microsomes. *Biochem. Pharmacol.* **1983**, *32*, 3419-3428.

[10] a) Laborde J, Deraeve C, Duhayon C, Pratviel G, Bernardes-Génisson V. The first biomimetic approach for ethionamide activation and mechanistic understanding of its into active and non active metabolites. *Org. Biomol. Chem.* **2016**, *14*, 8848-8858. b) Chipso K, Logan IE, Eskew MW, Omondi B, Simoyi RH. Kinetics and Mechanism of Bioactivation via *S*-oxygenation of anti-tubercular agent ethionamide by peracetic acid. *J. Phys. Chem. A* **2016**, *120*, 8056-8064.

[11] Decker CJ, Doerge DR, Cashman, JR. Metabolism of Benzimidazoline-2-thiones by rat hepatic microsomes and hog liver flavin-containing monooxygenase. *Chem. Res. Toxicol.* **1992**, 726-733.

[12] Olojo R, Simoyi RH. Oxyhalogen-sulfur chemistry: kinetics and mechanism of the oxidation of thionicotinamide by peracetic acid. *J. Phys. Chem. A* **2004**, *108*, 1018-1023.

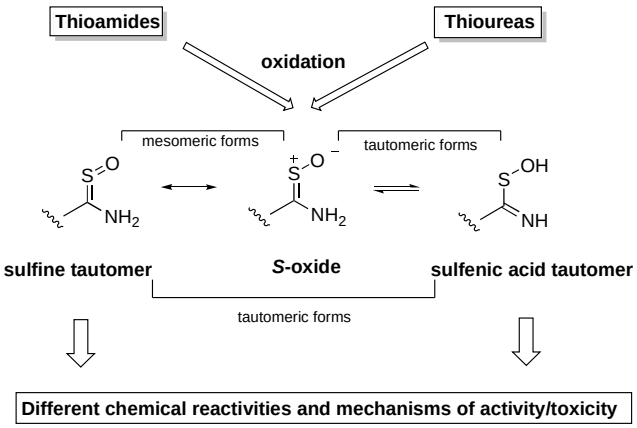
[13] a) Sarma D, Hajovsky H, Koen YM, Galeva NA, Williams TD, Staudinger JL et al. Covalent modification of lipids and proteins in rat hepatocytes and in vitro by thioacetamide metabolites. *Chem. Res. Toxicol.* **2012**, *25*, 1868-1877. b) Ji T, Ikehata K, Koen YM, Esch SW, Williams TD, Hanzlik RP. Covalent modification of microsomal lipids by thiobenzamide metabolites in vivo. *Chem. Res. Toxicol.* **2007**, *20*, 701-708.

[14] Buechter DD. Free radicals and oxygen toxicity. *Pharm. Res.* **1988**, *5*, 253-260.

[15] a) Poulsen LL, Hyslop RM, Ziegler DM. *S*-oxidation of thioureylenes catalyzed by a microsomal flavoprotein mixed-function oxidase. *Biochem. Pharmac.* **1974**, 3431-3440. b)

Crivas S, Ronne E. Facile desulfurization of cyclic thioureas by hydrogen peroxide in acetic acid. *Acta Chemica Scandinavica* **1995**, *49*, 225-229.
[16] a) Henderson MC, Krueger SK, Stevens JF and Williams DE. Human flavin-containing monooxygenase form 2 *S*-oxygenation: sulfenic acid formation from thioureas and oxidation of glutathione. *Chem. Res. Toxicol*. **2004**, *17*, 633-640. b) Decker CJ, Doerge DR. Rat hepatic microsomal metabolism of ethylenethiourea. Contributions of the flavin-containing monooxygenase and cytochrome P-450 isozymes. *Chem Res Toxicol*. **1991**, *4*, 482-489**.**
[17] a) Gaussian 09, Revision D.01, Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR et al. Gaussian, Inc., Wallingford CT, 2013. b) Zhao Y. and Truhlar DG. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc*. **2008**, *120*, 215-41. c) Tomasi J, Mennucci B, and Cammi R. Quantum mechanical continuum solvation models. *Chem. Rev*. **2005**, *105*, 2999-3093.

ANTITUBERCULAR DRUGS



Different chemical reactivities and mechanisms of activity/toxicity