Archives of Biochemistry and Biophysics xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

Sulfenic acids as reactive intermediates in xenobiotic metabolism

Daniel Mansuy *, Patrick M. Dansette *

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, Université Paris Descartes, 45 rue des Saints-pères, 75270 Paris Cedex 06, France

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Sulfenic acid
Xenobiotics
Metabolism
Cytochrome P450
Oxidation
Reactive metabolites

ABSTRACT

Sulfenic acid reactive intermediates are formed during the oxidation of cysteine residues of proteins and play key roles in enzyme catalysis, redox homeostasis and regulation of cell signalling. However few data are presently available on the formation and fate of sulfenic acids as reactive intermediates during the metabolism of xenobiotics. This article is a review of the xenobiotic metabolism situations in which the intermediate formation of a sulfenic acid has been reported. Formation of these intermediates has been either proposed on the basis of the isolation of products possibly deriving from sulfenic acids or shown after trapping of the sulfenic acid by specific nucleophiles. This review indicates the different mechanisms by which a sulphur-containing xenobiotic can be metabolized with the intermediate formation of a sulfenic acid. It also indicates the different possible fates of these sulfenic acids that have been reported in the literature. Finally, it discusses the possible implications of the formation of xenobiotic-derived sulfenic acid reactive metabolites in pharmacology and toxicology.

Introduction

Sulfenic acid reactive intermediates are formed during the oxidation of cysteine residues of proteins [1–6]. This oxidation of cysteines can be reversed through the action of specific enzymes such as thioredoxins. Thus, oxidation of specific protein cysteine residues may operate like a switch through the activation or deactivation of their cellular functions [5,6]. Some of those proteins are involved in signal transduction and many data suggest that sulfenic acids play key roles in enzyme catalysis, redox homeostasis and regulation of cell signalling [5,6].

Sulfenic acids often undergo further oxidations to more stable oxidized metabolites such as sulfinic and sulfonic acids (Eq. (1)).

$$\underset{\text{thiol}}{RSH} \longrightarrow \underset{\text{sulfenic acid}}{RSOH} \longrightarrow \underset{\text{sulfinic acid}}{RSO_2H} \longrightarrow \underset{\text{sulfonic acid}}{RSO_3H} \quad (1)$$

Cysteine sulfenic acids are highly reactive intermediates that could not be observed as such in most cases. However, in rare cases, they can be isolated and stabilized within particular protein micro-environments, and have been observed by X-ray crystallography and NMR spectroscopy [5]. In most cases, the formation of protein cysteine sulfenic acids has been shown through the detection of their stable adducts with specific nucleophiles. The most widely used trapping agent for sulfenic acids is dimedone, a C-nucleophile that rapidly reacts with the electrophilic sulphur atom of sulfenic acids with formation of stable adducts involving a C–S bond (Fig. 1) [7,8]. Another trapping agent often employed for the detection of protein sulfenic acids is 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)[1] (Fig. 2) [5,9].

Few data are presently available on the formation and fate of sulfenic acid reactive intermediates during the metabolism of xenobiotics [10–12]. As reactive electrophilic species they can covalently bind to cell nucleophiles such as proteins and be involved in pharmacological or toxicological effects of the parent xenobiotic. This article is a review of the situations in which the intermediate formation of a sulfenic acid has been either proposed or shown in the metabolism of xenobiotics. It indicates the different mechanisms by which a sulphur-containing xenobiotic can be metabolized with the intermediate formation of a sulfenic acid. It also indicates the different possible fates of these sulfenic acids that have been reported in the literature. Finally, it discusses the possible implications of the formation of xenobiotic-derived sulfenic acid reactive metabolites in pharmacology and toxicology.

Different ways of formation of sulfenic acid reactive Intermediates during xenobiotic metabolism

Formation of sulfenic acids by oxidation of a thiol function

Thiols, RSH, are good substrates of cytochrome P450- and flavin-dependent monooxygenases [10,13]. Thiols exhibiting a marked nucleophilic character of their S-atom are generally good substrates of flavin-dependent monooxygenases, whereas the less

* Corresponding authors. Fax: +33 142868387 (D. Mansuy).
E-mail addresses: daniel.mansuy@parisdescartes.fr (D. Mansuy), patrick.dansette@parisdescartes.fr (P.M. Dansette).

[1] *Abbreviations used:* NBD-Cl, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole; GSH, glutathione; Ticlid, Ticlopidine; TZD, thiazolidinedione; CYP102, cytochrome P450 BM_3.

0003-9861/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2010.09.015

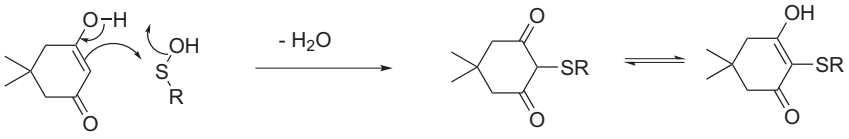


Fig. 1. Trapping of sulfenic acids with dimedone.

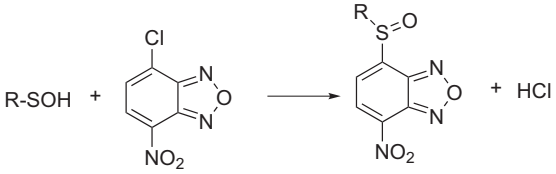


Fig. 2. Trapping of sulfenic acids by 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole.

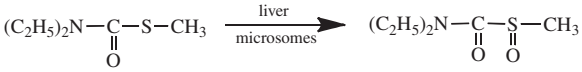
marked the nucleophilic character of the S-atom, the greater the probability of its oxidation by cytochromes P450 [10]. It is generally admitted that monooxygenase-catalyzed oxidation of thiols first leads to a sulfenic acid intermediate which can be further oxidized to the corresponding sulfinic acid and then to a sulfonic acid. However, sulfenic acids are electrophilic intermediates that rapidly react with the parent thiol RSH or another thiol present in the medium, such as glutathione (GSH), with formation of disulfides RSSR or RSSG [10] (Fig. 3).

Spironolactone **1**(Fig. 4) is an antimineralocorticoïd, diuretic drug containing a thioester $SCOCH_3$ function. The major metabolic route of **1** is a hydrolytic deacetylation leading to the corresponding thiol **2**. In the presence of liver microsomes, NADPH and O_2, the thiol function of **2** is oxidized with formation of the sulfinic and sulfonic acid metabolites **4** and **5**, that have been identified by HPLC-MS by comparison with authentic chemically synthesized compounds [14]. In the presence of GSH, another metabolite, the glutathionyl-spironolactone mixed disulfide **6** is formed [15] (Fig. 4). Moreover, during cytochrome P450-dependent oxidation of **2**, some cytochromes P450 are inactivated in a suicidal manner [14,16]. Metabolites **4**, **5** and **6** have been proposed to derive from a common precursor, sulfenic acid **3** that may either react with GSH with formation of **6** or undergo further oxidation with formation of **4** and **5**. Inactivation of cytochromes P450 during oxidation of **2** has been related to the formation of the electrophilic sulfenic acid **3**, or of a thiyl radical that could be an intermediate in the P450-catalyzed oxidation of thiol **2** into sulfenic acid **3** [14].

Formation of sulfenic acids during the oxidative metabolism of thioesters R–CO–S–R'

Plausible pathway for sulfenic acid formation from metabolic oxidation of thioesters

The high-valent iron-*oxo* active species involved in the catalytic cycle of dioxygen activation by cytochromes P450 should be able to transfer its oxygen atom to the S atom of thioesters RCOSR' with formation of the corresponding thioester S-oxides RCOSOR'. Actually, as we will see later, the formation of such a RCOSOR' species, with $R=N(C_2H_5)_2$ and $R'=CH_3$, has been shown during the oxidation of a disulfiram metabolite by liver microsomes [17] (Eq. (2)).



(2)

In general, the carbon atom of the carbonyl group of these thioester S-oxides should be highly electrophilic and should rapidly react with nucleophiles. Its reaction with H_2O should lead to a hydrolytic cleavage of the CO–SO bond with formation of RCOOH and a sulfenic acid intermediate R'SOH (Fig. 5). In the particular case of the disulfiram metabolite, the presence of the electron-donating substituent $N(C_2H_5)_2$ should decrease the reactivity of the carbonyl group of $(C_2H_5)_2NCOSOCH_3$ towards H_2O, explaining the relative stability and successful isolation of this oxidized metabolite [17].

Recent evidence for the formation of sulfenic acid reactive intermediates during the metabolism of the anti-thrombotic drugs ticlopidine, clopidogrel and prasugrel

Ticlopidine (Ticlid) **7a** and clopidogrel (Plavix, Iscover) **7b** (Fig. 6) are anti-thrombotic prodrugs of the tetrahydrothienopyridine series that must be metabolized *in vivo* into the corresponding pharmacologically active thiols **9a** and **9b**, respectively, to exert their activity as antagonists of the platelet receptor P2Y12 [18–20]. Ticlopidine was introduced to the market in 1979 for prevention of thrombotic stroke. Clopidogrel was first launched in 1998 in the USA and in 1999 in Europe, for the reduction of atherosclerotic problems in patients with stroke, myocardial infraction or peripheral arterial disease. Their metabolic activation occurs in two steps that are catalyzed by cytochromes P450 [18,19]. The first step is a classical cytochrome P450-dependent hydroxylation of the thiophene ring by NADPH and O_2 leading to thiolactone metabolites **8a** and **8b**, respectively [21–23] (Fig. 6). It is mainly catalyzed by CYP 2C19 and CYP 2B6 in the case of **8a** and by CYP 2C19, CYP 1A2 and CYP 2B6 in the case of **8b** [24]. The second step leading to the pharmacologically active thiols **9a** and **9b** also requires the involvement of a cytochrome P450-catalyzed oxidative step with consumption of NADPH and O_2 (CYP 3A4, CYP 2B6, CYP 2C9 and CYP 2C19 are mainly involved in oxidation of **8b**) [24,25]. It has been recently shown that this step is an oxidative cleavage of the

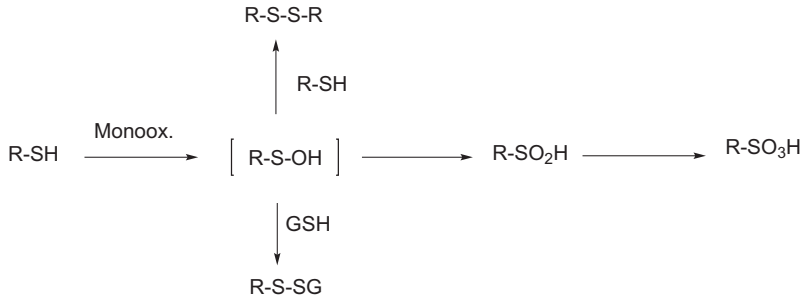


Fig. 3. Different possible products formed upon metabolism of xenobiotic thiols.

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015

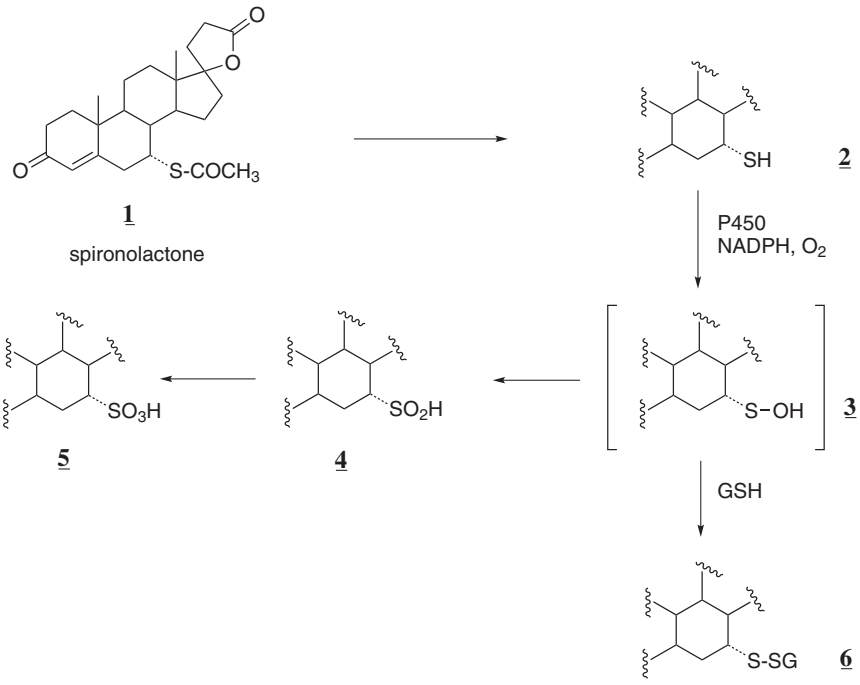


Fig. 4. Proposed intermediate formation of a sulfenic acid during metabolism of spironolactone.

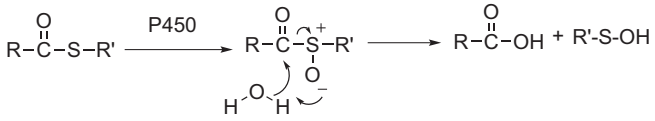


Fig. 5. Plausible mechanism for sulfenic acid formation during oxidative metabolism of thioesters.

thiolactone ring of metabolite **8** leading to the formation of reactive sulfenic acid intermediates **10** [26]. These intermediates have been trapped by dimedone during oxidative metabolism of thiolactones **8** by rat and human liver microsomes or by several recombinant human liver cytochromes P450, namely CYP 3A4, CYP 2C8, CYP 2C9, CYP 1A2 and CYP 2B6. The corresponding dimedone adducts **11** have been completely characterized by NMR spectros-

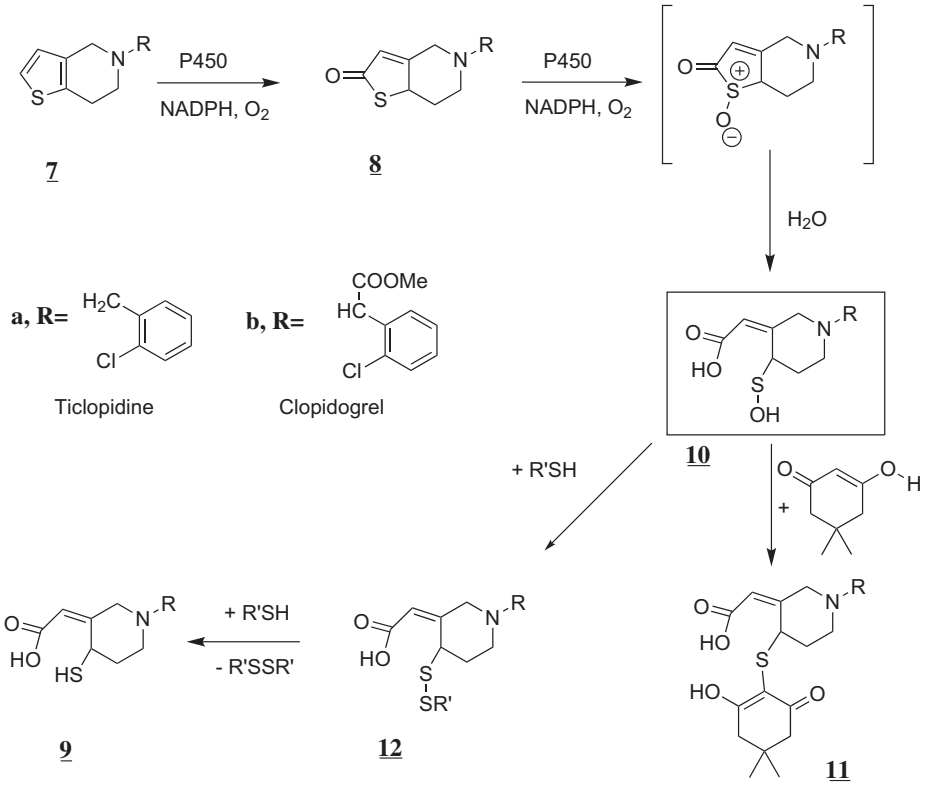


Fig. 6. Intermediate formation of a sulfenic acid during metabolic activation of ticlopidine **7a**, and clopidogrel **7b**. The sulfenic acid intermediates have been trapped by dimedone or thiols (R'SH=mercaptoethanol, N-acetylcysteine and glutathione).

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015

copy and mass spectrometry [26]. Sulfenic acid intermediates have also been trapped by thiols added to the incubation medium, such as mercapto-ethanol, N-acetylcysteine and glutathione. The resulting mixed disulfides **12** (Fig. 6) have been characterized by mass spectrometry [26]. In the presence of a large excess of R'SH thiol, disulfides **12** are further converted to the pharmacologically active metabolites **9** [18,19,24] whose formation was shown *in vivo* in man [24]. These data [26] have provided the first evidence for the formation of sulfenic acid reactive intermediates in the metabolism of tetrahydrothienopyridine anti-thrombotic drugs, and, in a more general manner for the formation of a sulfenic acid in cytochrome P450-catalyzed oxidative cleavage of thioesters, according to Fig. 5. They have also suggested a first detailed pathway for the formation of active thiols **9** in the metabolism of ticlopidine and clopidogrel. This pathway would involve two successive cytochrome P450-dependent monooxygenations leading to sulfenic acids **10**, followed by a reduction of **10** to pharmacologically active **9** due to two successive reactions of a thiol, which should be GSH *in vivo*, with intermediate formation of a disulfide **12** (Fig. 6).

Prasugrel (Effient) **13** is the newest member of the class of tetrahydrothienopyridine anti-thrombotic prodrugs. It is also an irreversible inhibitor of the platelet receptor P2Y12, after metabolic conversion *in vivo* to a pharmacologically active 4-mercapto-3-piperidinyliden acetic acid derivative **15**, that is analogous to **9** [27,28] (Fig. 7). Metabolic conversion of prasugrel to **15** involves two enzymatic reactions: (i) the hydrolysis of its ester function leading to thiolactone **14**, which seems to be mainly catalyzed by the hCE_2 enzyme in man [29], and (ii) the oxidative cleavage of the thioester bond of **14** with the eventual formation of **15**, which is catalyzed by cytochromes P450 3A4, 2B6, 2C9 or 2C19 in man [24]. As in bioactivation of ticlopidine and clopidogrel, it has been shown that this cytochrome P450-catalyzed oxidative cleavage of the thioester bond of thiolactone **14** led to the formation of an intermediate sulfenic acid **16** [30,31]. Formation of **16** was demonstrated by trapping it with dimedone in incubations of prasugrel with rat or human liver microsomes in the presence of NADPH [31]. The resulting stable adduct **17** was isolated and completely characterized by mass spectrometry and detailed NMR spectroscopy [31]. In the presence of thiols such as GSH [30,31] or 2-nitro-5-thiobenzoic acid [30], sulfenic acid **16** was trapped with formation of mixed disulfides such as **18**. In the presence of the water-soluble phosphine, $P(CH_2CH_2COOH)_3$, that has been previously used to reduce sulfenic acids [32], incubation of prasugrel with liver microsomes and NADPH led to thiol **15** [31]. These data indicate that sulfenic acid **16** can be efficiently trapped by C-nucleophiles such as dimedone, S-nucleophiles such as GSH or simpler thiols, and P-nucleophiles such as $P(CH_2CH_2COOH)_3$. A possible mechanism for the last reaction is shown in Fig. 8 [31]. Trapping of sulfenic acid intermediates by dimedone during metabolism of xenobiotics *in vitro* appears as the best method of detection of such intermediates because the corresponding adducts, that involve a stable C–S bond, are generally stable in the metabolic incubation media. Trapping of sulfenic acids by the water-soluble phosphine $P(CH_2CH_2COOH)_3$ seems to be a clean method for the eventual formation of the thiol metabolites derived from sulfenic acids reduction. These thiol metabolites themselves can be trapped by N-ethyl-maleimide to form thioether adducts such as **20** that are more stable than their thiol precursors under metabolic conditions [31] (Fig. 7).

Trapping of sulfenic acids by thiols such as GSH leads to mixed disulfides such as **18.** However, thiols are less specific trapping agents of sulfenic acids than dimedone, because the obtained mixed disulfide adducts can be formed by various pathways that do not involve the intermediate formation of sulfenic acids. Moreover, mixed disulfides are much less stable adducts than the sulfenic acid-dimedone adducts, as they are very reactive towards thiols that could be present in the reaction media. This is illustrated by the results obtained during microsomal oxidations of ticlopidine, clopidogrel or prasugrel in the presence of GSH in excess which

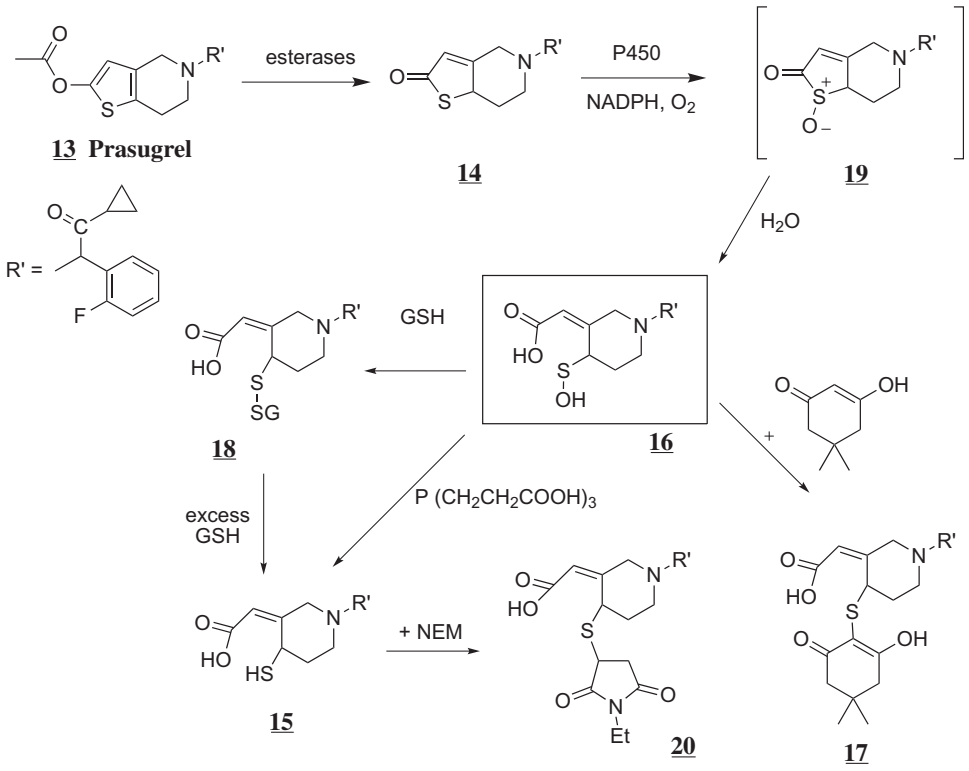


Fig. 7. Intermediate formation of a sulfenic acid during metabolic activation of prasugrel, **13**. The sulfenic acid intermediate has been trapped by dimedone, $P(CH_2CH_2COOH)_3$ or GSH. NEM = N-ethylmaleimide.

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015

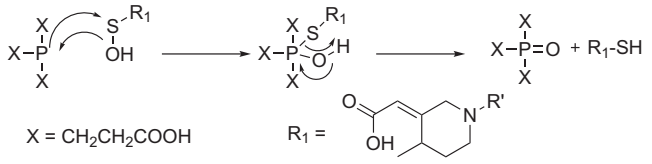


Fig. 8. Possible mechanism for the reduction of sulfenic acids by phosphines.

showed that the intermediate disulfide glutathionyl adducts, **12** and **18**, further react with GSH with the eventual formation of thiols **9** and **15**, respectively [26,30,31]. Anyway, trapping of sulfenic acids by GSH is an interesting reaction that provides cystathionyl mixed disulfides which could be intermediates and/or metabolites in the metabolic transformation of thiolactones such as **8** and **14** into thiols **9** and **15,** respectively.

Coming back to the oxidative cleavage of thiolactones such as **14** leading to sulfenic acids, it is likely that the first step of this reaction is a transfer of the oxygen atom of the high-valent iron-*oxo* (Fe=O) species of cytochrome P450 to the thiolactone sulphur atom, leading to a highly reactive thiolactone S-oxide **19** (Fig. 7). Three pathways could be considered for the following step, the hydrolytic cleavage of the CO–SO bond of this thiolactone S-oxide (Fig. 9)): (a) a nucleophilic attack of H_2O on its carbonyl group leading directly to **16** after protonation of the SO moiety, (b) a rearrangement of thiolactone S-oxide **19** coming from an intramolecular attack of the O^- atom on the CO group, with formation of a six-membered cyclic sulfenic ester **19'** (Fig. 9), followed by reaction of H_2O on the carbonyl group, or (c) the same rearrangement but followed by reaction of H_2O on the electrophilic sulphur atom of the six-membered sulfenic ester. In pathways a) and b) one of the two oxygen atoms of the carboxylate group of **16** is coming from H_2O, whereas, in pathway c), one of the oxygen atoms of the carboxylate group of **16** should come from O_2 during P450-dependent sulfoxidation of **14**. Incubation of prasugrel **13**, with rat liver microsomes in the presence of NADPH, dimedone and $^{18}O_2$ led to adduct **17**, that has not incorporated ^{18}O atoms (<5%). Identical incubations under $^{16}O_2$ but in $H_2{}^{18}O$ showed a major incorporation of ^{18}O (>85%) into the dimedone adduct. These experiments [31] indicated that the oxygen atom incorporated into adduct **17** came from water and not from O_2, which ruled out pathway (c) of Fig. 9 for sulfenic acid formation. Thus, formation of sulfenic acid **16** should involve a nucleophilic attack of H_2O on the electrophilic carbonyl group of either thiolactone S-oxide **19** or its rearranged sulfenic acid ester product **19'**. Very similar results were obtained in the same labelling experiments of incubation of **8b**, the thiolactone metabolite of clopidogrel with rat liver microsomes, NADPH, and dimedone (P.M. Dansette, S. Thébault and D. Mansuy, unpublished results).

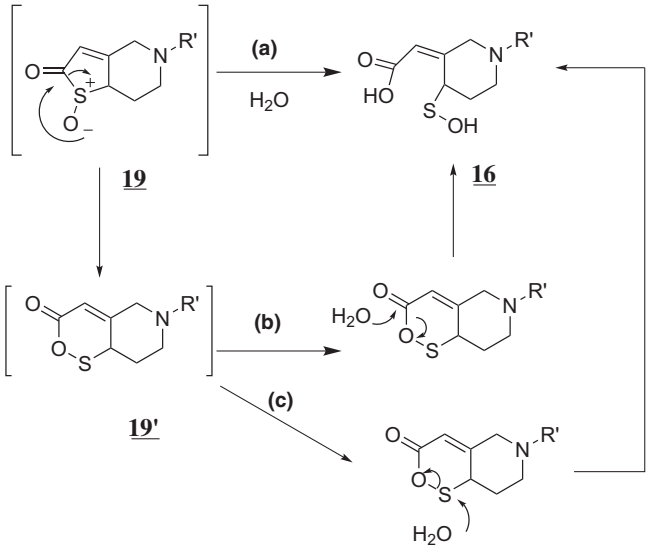


Fig. 9. Possible pathways for the formation of a sulfenic acid derived from prasugrel from the corresponding thiolactone S-oxide.

Indirect evidence for the formation of sulfenic acid intermediates during the metabolism of glitazones

Glitazones are thiazolidinedione (TZD)-containing drugs widely used in the treatment of type-2 diabetes. Several *in vitro* and *in vivo* studies have shown that the TZD ring of various glitazones such as troglitazone **21a**, MK-0767 **21b**, and MRL-A **21c** (Fig. 10), was subject to an oxidative metabolism resulting in its opening with formation of reactive metabolites [33–34]. A detailed study of the metabolites derived from the oxidation of the TZD ring of **21c** by monkey liver microsomes in the presence of NADPH showed the formation of several compounds that should derive from sulfenic acid **22c** [34] (Fig. 11). This includes glutathionyl adduct **23c**, that could result from the attack of the sulphur atom of GSH on the sulphur atom of **22c**, thiol **24c**, and sulfinic acid **25c**. It was proposed that the two last compounds could derive from a disproportionation of sulfenic acid **22c**, according to Fig. 12, and that thiol **24c** could also come from reduction of **22c** by NADPH or of **23c** by GSH in excess [34].

This mechanism proposed for the oxidative cleavage of the TZD ring of glitazones is very similar to that described above for the oxidative cleavage of the lactone ring of ticlopidine, clopidogrel and prasugrel. In both cases, the reaction should start with an S-oxidation of the ring followed by a fast hydrolytic opening upon attack of H_2O on the very electrophilic carbon of the intermediate. In the case of glitazones, another possible fate of this intermediate has been proposed [34], the β-elimination of the NH proton leading to a very reactive isocyanate that would react with H_2O to give the corresponding amide after loss of CO_2 (Fig. 11). The fates of the intermediate sulfenic acids were similar for the two series of drugs, with the formation of the corresponding thiols and glutathione mixed disulfide adducts in both cases. The main difference was concerned with the formation of a sulfinic acid derived from glitazones [33,34] that has not been observed so far in the case of the tetrahydrothienopyridine drugs.

Another mode of metabolic activation of the TZD ring was proposed on the basis of the detection and characterization by HPLC-MS of another GSH adduct **26a** upon oxidation of troglitazone by rat liver microsomes [35] (Fig. 13). Formation of this adduct was interpreted as a reaction of GSH on an intermediate sulfenium

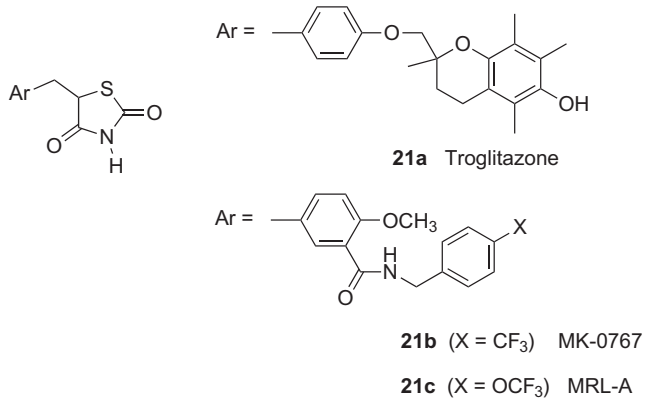


Fig. 10. Formula of glitazones MK-0767, MRL-A and troglitazone.

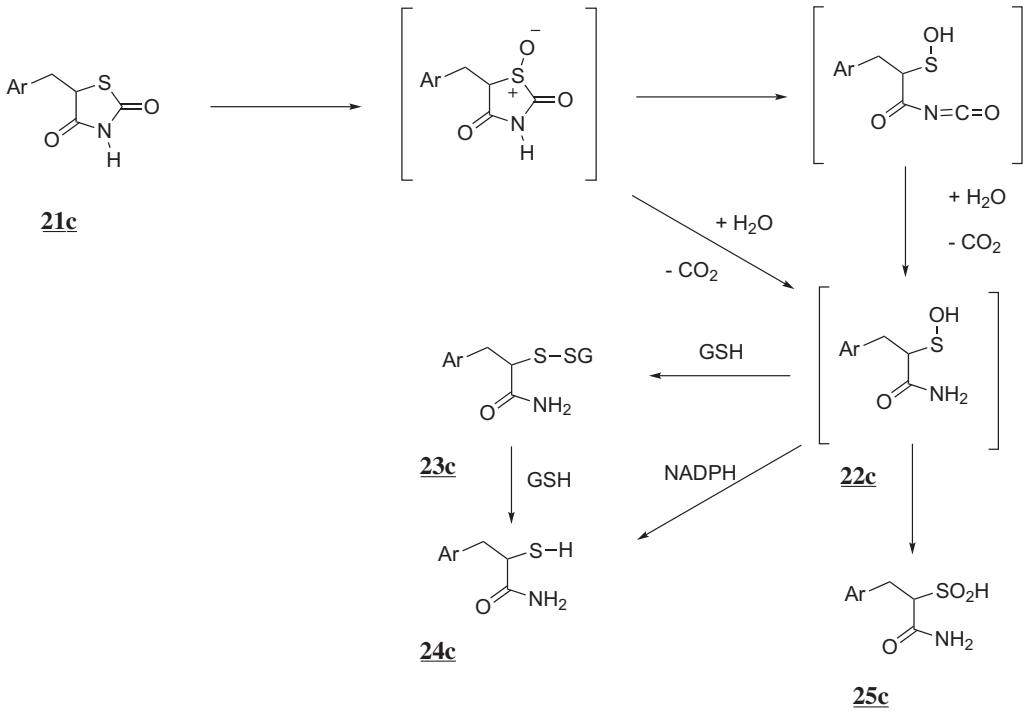


Fig. 11. Proposed formation of a sulfenic acid intermediate during metabolic activation of **21c**.

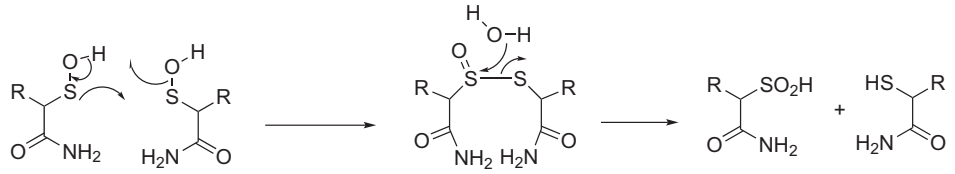


Fig. 12. Proposed mechanism of disproportionation of sulfenic acid **22c**.

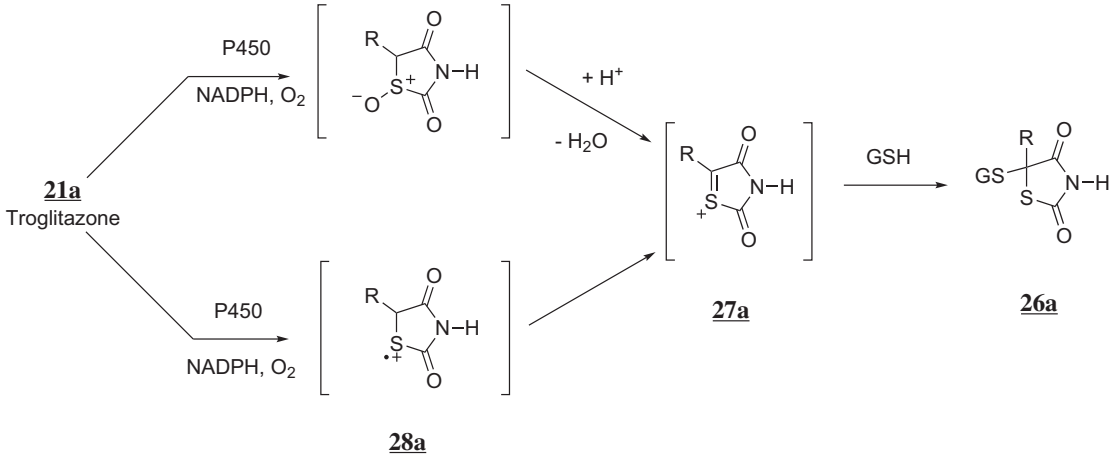


Fig. 13. Mechanism proposed for the formation of a glutathione adduct upon S-oxidation of troglitazone.

ion, **27a**, that could come either from a loss of H_2O from the S-oxide intermediate or from the sulphur radical-cation **28a**.

Possible formation of sulfenic acid intermediates during the metabolism of thio- and dithio-carbamates related to disulfiram

Disulfiram (Antabuse) (Fig. 14), that is used in the clinical treatment of alcoholism, exerts its pharmacological effects as an inhibitor of liver mitochondrial alcohol dehydrogenase. These inhibitory effects are due to electrophilic metabolites of disulfiram [17,36–38]. Thiocarbamate **29** is a metabolite of disulfiram involving a CO–S bond, that undergoes an S-oxidation, as the thioesters discussed above (see general Fig. 5). The resulting metabolite **30** that involves a CO–SO bond is the equivalent of the intermediate S-oxide formed upon metabolic activation of thioesters related to

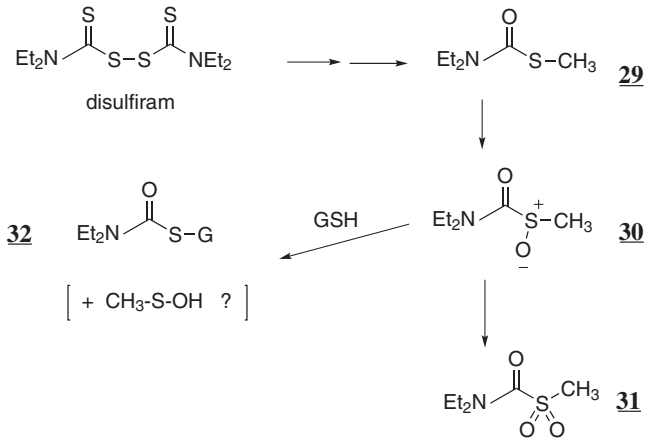


Fig. 14. Metabolic activation of disulfiram leading to a thioester and its various electrophilic metabolites.

anti-thrombotic tetrahydrothienopyridines and to glitazones. However, in this case, metabolite **30** is stable enough to be isolated; its formation has been completely established, and an authentic sample of N,N-diethylthiolcarbamate S-oxide, **30**, has been synthesized from oxidation of **29** with one equivalent of $NaIO_4$ [17]. Thiols such as GSH or N-acetylcysteine react with the electrophilic carbonyl carbon of **30** with formation of thiol adducts such as the acylglutathione compound **32** that was detected in the bile of rats treated with disulfiram [36,37]. In this reaction of GSH with **30**, formation of **32** should be accompanied with the formation of methyl sulfenic acid (Fig. 14). However, the formation of this sulfenic acid remains to be established, and the irreversible inhibition of alcohol dehydrogenase by disulfiram metabolites is believed to derive from reactions of this protein with electrophilic metabolites such as **30**, **31**, and their equivalents involving a C=S moiety [36,37].

Certain thio- and dithio-carbamate herbicides and pesticides, which also inhibit alcohol dehydrogenase *in vivo*, have been shown to form electrophilic S-oxidized metabolites similar to those proposed for disulfiram [39–41].

Possible formation of sulfenic acid intermediates during irreversible inhibition of cytochrome P450 BM_3 by fatty acid thioesters

Fatty diacid monoethyl thioesters are irreversible inhibitors for cytochrome P450 BM_3 (CYP102), that exploit the ω-2 oxidation specificity of this cytochrome [42]. Catalytic oxidation of the monoethyl thioesters of dodecanedioic and hexadecanedioic acids results in cytochrome P450 BM_3 inactivation and formation of the parent diacids as metabolites [42]. GSH prevents this inactivation of the enzyme, and it has been proposed that an electrophilic metabolite is responsible for enzyme inactivation. Thioester S-oxide **33** has been proposed as such an electrophilic metabolite (Fig. 15). Reaction of its carbonyl carbon with H_2O should lead to the eventual diacid metabolite, whereas its reaction with a nucleophilic residue of the protein should be at the origin of enzyme inactivation. During these reactions, the SEt part of **33** should be released as the EtSOH sulfenic acid. However, formation of this sulfenic acid remains to be demonstrated.

Other examples of oxidative metabolism of thioesters with intermediate formation of reactive thioester S-oxides

The herbicidal compound dithiopyr **34** contains two thioester functions (Fig. 16). Its transformation by rat liver microsomes leads to the corresponding carboxylic acids such as **35** as main metabolites [43]. This transformation of its thioester functions into carboxylic acids has been found to be catalyzed by monooxygenases, and not esterases. Microsomal metabolism of dithiopyr also leads to a glutathione conjugate **36** (Fig. 16) globally resulting from the replacement of the SCH_3 group of a dithiopyr thioester function with the SG group of glutathione. On the basis of those results, the mechanism of transformation of the thioester functions of dithiopyr into carboxylic acid functions was proposed to proceed through a monooxygenase-catalyzed S-oxidation followed by a hydrolysis of the very reactive CO–SO bond of the intermediate **37** (Fig. 16).

The anti-inflammatory glucocorticoid drug, fluticazone propionate **38**, also involves a thioester function. Its main metabolite in man is the carboxylic acid **39** formally deriving from the hydrolysis of its thioester function [44] (Fig. 17). However, it was also found in that case that this reaction involved an oxidation mainly catalyzed by the cytochromes P450 of the 3A subfamily [44].

Another anti-inflammatory steroid drug involving a thioester function, tixocortol pivalate **40** (Fig. 17), led to several metabolites in man, such as **41** and **42**, resulting from a cleavage of its thioester bond and its replacement with $S–CH_3$ and SO_2CH_3 functions [45]. The detailed mechanisms of the cleavage of the thioester function of fluticazone propionate and tixocortol pivalate have not been determined. However, a mechanism similar to that found in the case of dithiopyr would involve a monooxygenase-catalyzed S-oxidation of their thioester function followed by a hydrolysis of the CO–SO bond of the intermediate S-oxide. The sulfenic acid metabolites resulting from this hydrolysis have not been looked for. Formation of such a sulfenic acid intermediate in the case of tixocortol would explain the formation of the thioether and sulfone metabolites from further reactions of reduction, methylation and S-oxidation, that have been previously found during metabolism of prasugrel and glitazones [24,33].

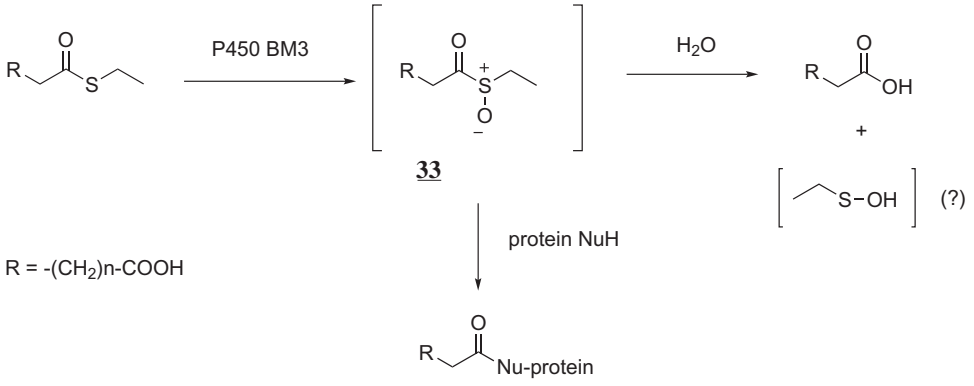


Fig. 15. Mechanism proposed for the metabolic activation of fatty acid thioesters used as irreversible inhibitors of P450 BM_3.

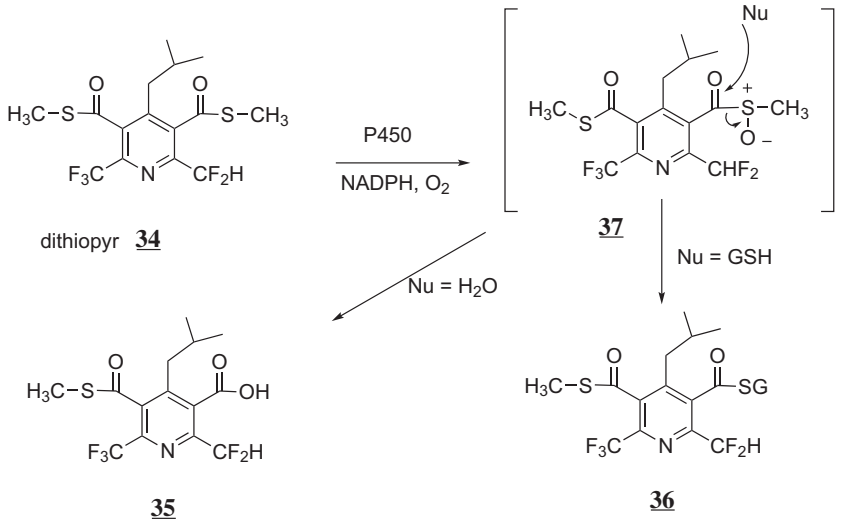


Fig. 16. Formation of a reactive intermediate during metabolic transformation of the thioester functions of dithiopyr into carboxylic acid functions.

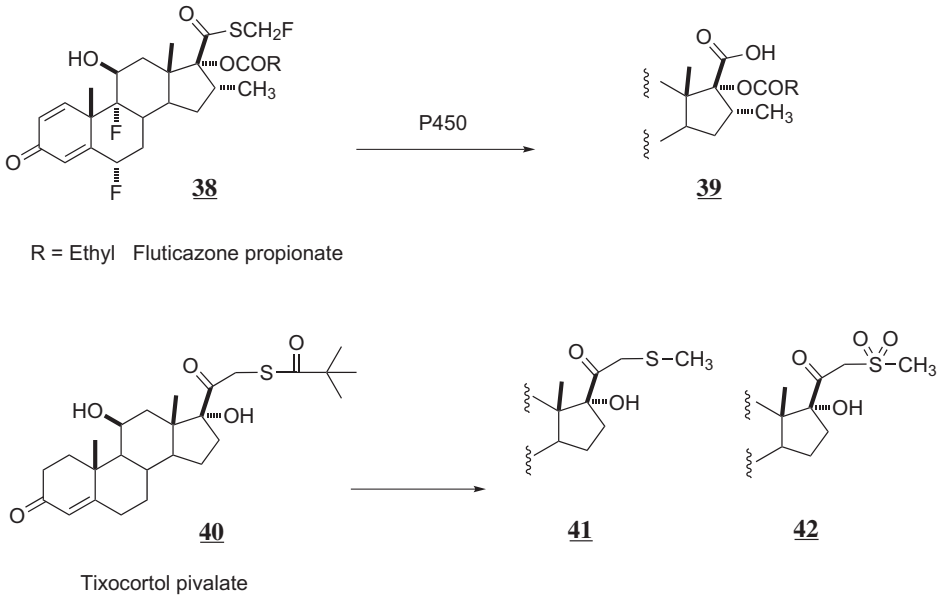


Fig. 17. Metabolism of the thioester functions of fluticasone propionate and tixocortol pivalate in man.

Formation of sulfenic acids from metabolism of sulfoxides

Formation of sulfenic acids during metabolic activation of H^+/K^+ATPase inhibitors such as omeprazole

Drugs, such as omeprazole, esomeprazole and lansoprazole, that are used to treat gastreso–phageal reflux disease, act as inhibitors of the proton transport by the gastric H^+/K^+ ATPase. Acutally, these compounds are produgs that must be activated *in situ* into reactive metabolites able to bind covalently to cysteine residues of H^+/K^+ ATPase [46–48]. The mechanism proposed for this activation is shown in Fig. 18 in the case of omeprazole **43**. According to this mechanism, this prodrug is activated under the acidic conditions of the stomach with intermediate formation of a spiro intermediate **44** that undergoes aromatization with cleavage of a C–S bond and formation of sulfenic acid **45**. This sulfenic acid, or sulfeneamide **46** that derives from its intramolecular reaction with a benzimidazole nitrogen atom, reacts with a cysteine of H^+/K^+ ATPase to form a disulfide adduct **47**. It is noteworthy that reactive electrophilic intermediates **45** and **46** are only formed *in situ* in close proximity of the target after protonation of **43** under the stomach acidic conditions.

Interestingly, a sulfenic acid intermediate would be formed during metabolism of pantoprazole **48**, a proton pump inhibitor of the prazole series, from an addition–elimination reaction of GSH on the C-2 atom of the benzimidazole moiety of **48** (Fig. 19). Such a reaction should lead to sulfenic acid **50** and the glutathione conjugate **49**. Another GSH conjugate and another intermediate sulfenic acid **52** would be formed from a nucleophilic attack of GSH on the activated benzylic CH_2 group of pantoprazole, resulting in a cleavage of its CH_2-SO bond and formation of a benzylic GSH adduct **51** and sulfenic acid **52** [49]. Actually, the two GSH adducts derived from pantoprazole, **49** and **51**, were identified at the level of their further breakdown products [49].

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015

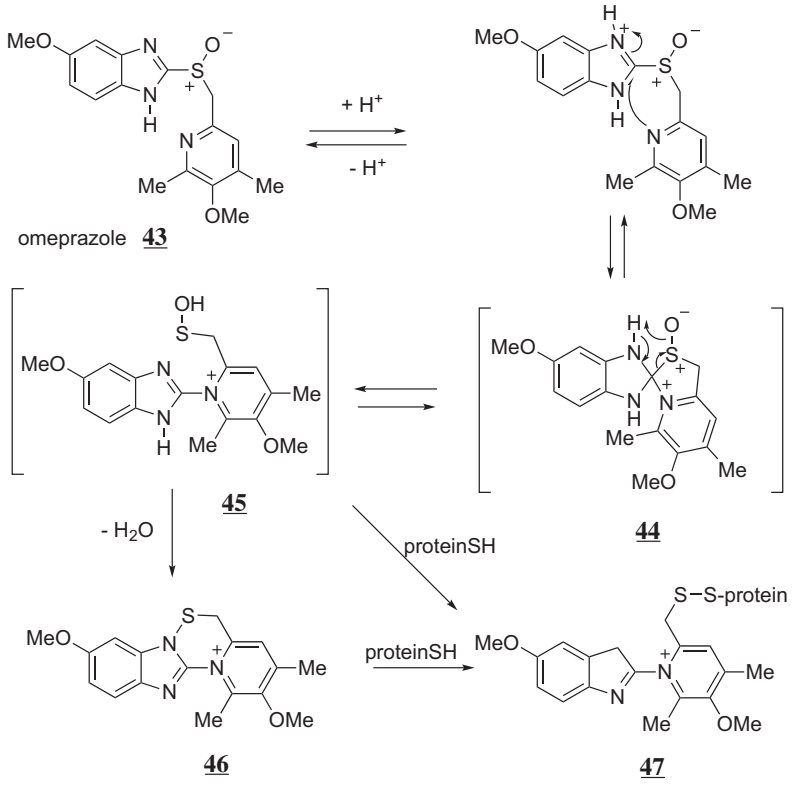


Fig. 18. Proposed formation of a sulfenic acid during metabolic bioactivation of omeprazole.

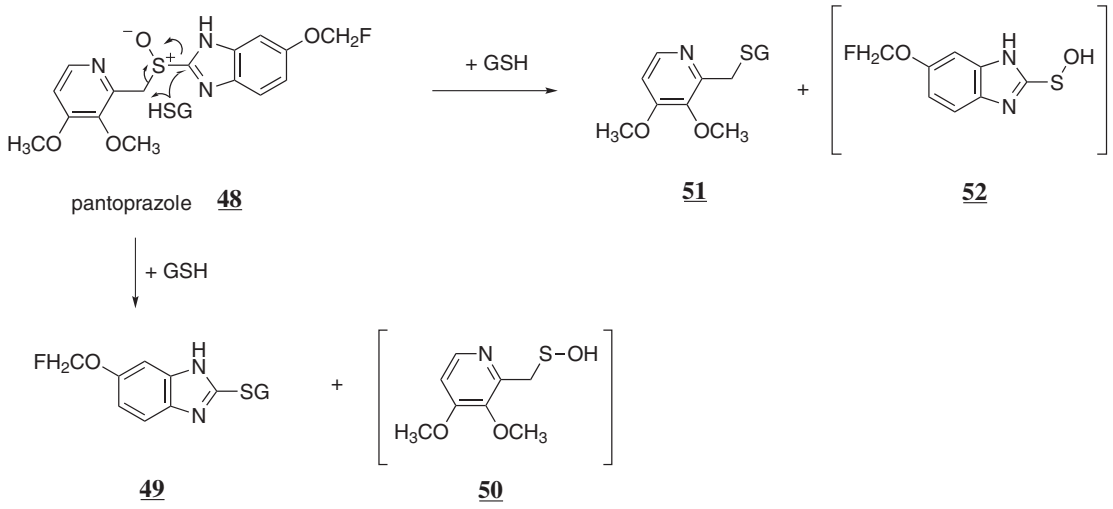


Fig. 19. Proposed formation of sulfenic acids during metabolism of pantoprazole.

Intermediate formation of a sulfenic acid in the metabolism of an allylic sulfoxide

Diallyl sulfide, a flavour component of garlic, is metabolized in rats into many compounds deriving from an initial S-oxidation followed by glutathione conjugation [50]. Reaction of diallyl sulfoxide **53** with GSH leads to two GSH conjugates, **54** and **55**, and sulfenic acid **56**. This sulfenic acid was not characterized as such, but its formation was supported by the isolation of allyl-SSG, **55**, a product expected from reaction of **56** with GSH (Fig. 20).

Conclusion

Different chemical situations leading to sulfenic acid intermediates during xenobiotic metabolism, fate of these intermediates in biological media, and consequences of their formation in pharmacology and toxicology

From the presently available literature data concerning sulfenic acid formation during xenobiotic metabolism, that are described in the previous sections, it appears that such a formation was

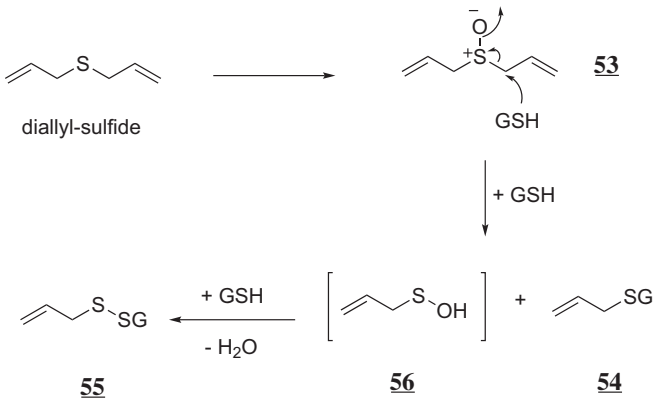


Fig. 20. Proposed formation of a sulfenic acid during metabolism of diallyl sulfide.

established by specific trapping in very few cases. Sulfenic acid formation was most often proposed on the basis of the isolation of metabolites that could derive from them, such as glutathionyl adducts RSSG, sulfinic and sulfonic acids RSO_2H and RSO_3H, or thiols RSH.

These literature data indicate that sulfenic acid formation during xenobiotic metabolism occurs in three chemical situations depending on the xenobiotic structure: (i) the S-hydroxylation of xenobiotics involving a thiol function, (ii) the S-oxidation of thioesters RCOSR' leading to an oxidative cleavage of their CO–S bond, and (iii) the cleavage of a C–S bond of xenobiotics bearing a sulfoxide function, resulting from the attack of a nucleophile (GSH for instance) on an electrophilic carbon in α-position of the SO function. Actually, if one excepts the S-hydroxylation of thiols, most of the above described (or proposed) sulfenic acid formations are derived from a common general reaction, the attack of a nucleophile (H_2O or GSH) on an electrophilic carbon in α-position of a sulfoxide function that is present either in the starting xenobiotic or in a metabolite. This nucleophilic attack results in the cleavage of the carbon-SO bond with formation of a sulfenic acid (Fig. 21A). This electrophilic carbon may be an allylic carbon in the case of diallylsulfoxide (Fig. 20), a benzylic carbon in the case of pantoprazole (Fig. 19), a carbonyl carbon in the case of the S-oxide metabolites of thioesters (Figs. 6 and 7), and a $C{=}N^+$ carbon in the case of omeprazole (Fig. 18). However, it is noteworthy that in the case of sulfenic acid formation from the oxidation of some thioesters, another mechanism proposed for the cleavage of a C–SO bond was based on the loss of a β-proton (relative to the SO function) resulting in a 1,2-elimination of RSOH (Fig. 21B). Such a mechanism has been proposed for sulfenic acid formation from glitazones [34] (Fig. 11) and fatty acid thioesters [42].

The possible fates of sulfenic acid intermediates RSOH formed during xenobiotic metabolism that can be derived from the presently available literature data are summarized in Fig. 22. Most often, they derive from reactions with nucleophiles. Reactions with thiols R'SH lead to mixed disulfides RSSR' or to the thiol RSH derived from RSOH if R'SH is used in excess. In that regard, the most relevant reactions in xenobiotic metabolism are those involving GSH, the detection of glutathionyl adducts RSSG being a first indication of intermediate formation of sulfenic acids. Reaction with P-nucleophiles such as water-soluble phosphines appears to be an interesting method to transform sulfenic acid intermediates into more stable thiols (Figs. 7 and 8). Reaction with the C-nucleophile dimedone is a good method to efficiently trap sulfenic acid metabolites with formation of stable adducts (Figs. 6 and 7). Reaction with nucleophilic residues of proteins is responsible for the covalent binding of sulfenic acids to proteins that may be at the origin of pharmacological or toxicological effects of the parent xenobiotic.

Other fates of xenobiotic-derived sulfenic acid intermediates reported in the literature correspond to redox reactions occurring at the level of the S–OH function. Further oxidations lead to sulfinic and sulfonic acids that are much more stable than sulfenic acids and that have been isolated as stable metabolites of several xenobiotics (see for instance Figs. 4 and 11). Reduction of the sulfenic acid function to the corresponding thiol could occur by several pathways. From the presently available literature data, the most frequently found pathway is reduction by GSH with the intermediate formation of a glutathionyl adduct RSSG (see for instance Fig. 22 and the above discussion). Other thiols gave the same result

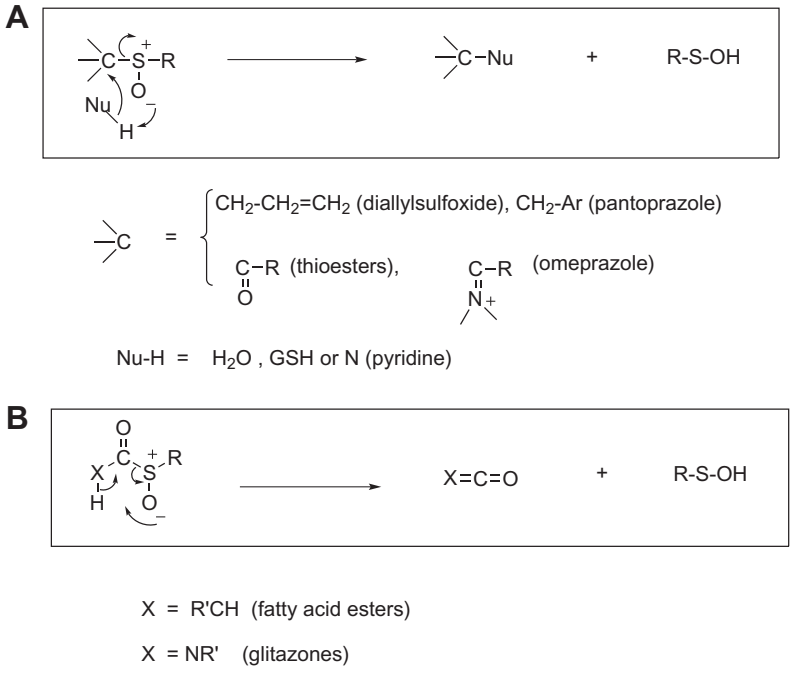


Fig. 21. Reactions of nucleophilic substitution (21A) or of β-elimination (21B) that are involved in all the proposed or demonstrated formations of sulfenic acids during xenobiotic metabolism (if one excepts the S-hydroxylation of thiols).

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015

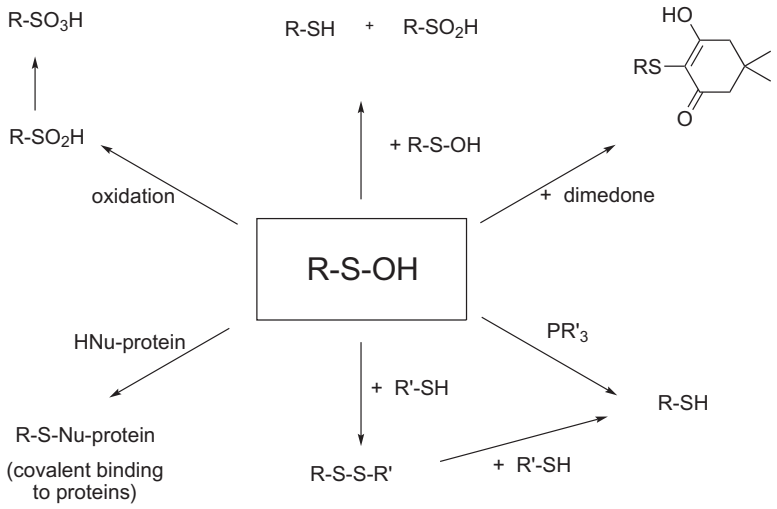


Fig. 22. Various possible fates of xenobiotic-derived sulfenic acid metabolites from literature data.

[26,30,31,34], and chemical reductants such as phosphines [31], AsO_2^-, and ascorbate (P. Dansette and D. Mansuy, unpublished results) were found to reduce the sulfenic acid intermediate derived from prasugrel into the corresponding thiol. Direct reduction with NADPH of sulfenic acid **22c**, that was proposed to derive from the oxidation of **21c** by recombinant P450 3A4 and cumyl hydroperoxide, also led to the corresponding thiol **24c** [34]. Protein sulfenic acid intermediates have been reported to be recycled back to the thiol state by several enzymatic systems [5]. However, no data are presently available on possible enzymatic reduction of xenobiotic-derived sulfenic acid intermediates. This should be mainly due to the chemical unstability of sulfenic acids that makes very difficult the study of their possible reduction by enzymatic systems.

Another possible way of formation of thiols RSH and sulfinic acids RSO_2H from sulfenic acids is the dismutation of sulfenic acids, as previously proposed for glitazone-derived sulfenic acids [34] (Fig. 12) and as shown for purine-6-sulfenic acid, a metabolite of the antineoplastic agent 6-thiopurine, that disproportionates to give 6-thiopurine and purine-6-sulfinic acid [51]. Formation of glutathionyl mixed disulfide adducts and/or sulfinic and sulfonic acid metabolites are a good indication but not a proof for intermediate formation of sulfenic acids. Trapping of these intermediates by dimedone and characterization of the corresponding stable dimedone adducts is a more direct evidence for their formation.

Formation of electrophilic sulfenic acid metabolites of xenobiotics should have important consequences in pharmacology and toxicology. However, few data are presently available on this subject in the literature, presumably because of the limited number of studies that have been published to establish the formation of sulfenic acids during xenobiotic metabolism and to discuss the fate of these intermediates. Reaction of sulfenic acid metabolites of omeprazole derivatives with cysteine residues of gastric H^+/K^+ ATPase appears to lead to an inhibition of this protein which is at the origin of the pharmacological effects of these "prasole" prodrugs (Fig. 18) [46–48]. Sulfenic acid intermediates are formed during metabolism of the anti-thrombotic prodrugs, ticlopidine, clopidogrel and prasugrel (Figs. 6 and 7) [26,30,31]. The thiol metabolites deriving from their reduction have been proposed as the pharmacologically active species that are responsible for the inhibition of the P2Y12 platelet receptor after formation of a covalent disulfide bond with a cysteine residue of this protein [20,52]. Another possible mechanism for this inactivation of the P2Y12 receptor could be the formation of such a covalent bond from reaction of their sulfenic acid metabolites **10** and **16**, or of the glutathionyl adducts **12** and **18** deriving from their reaction with GSH (Figs. 6 and 7) with a cysteine SH residue of the P2Y12 platelet receptor [26,31]. The latter mechanism remains to be established.

The inhibition of aldehyde dehydrogenase that is involved in the pharmacological effects of disulfiram appears to derive from a reaction of this enzyme with electrophilic metabolites of disulfiram such as **30** or **31** (Fig. 14) [17,36,37,53]. The sulfenic acid possibly formed in disulfiram metabolism (Fig. 14) could be another electrophilic species responsible for aldehyde dehydrogenase inactivation. Finally, cytochrome P450 inactivation observed during metabolic oxidation of spironolactone [14,54] (Fig. 4) appears to be due to electrophilic species formed by S-oxidation of the thiol metabolite, which could be the sulfenic acid itself or a thiyl radical intermediate [14].

Much less data are presently available about the role of xenobiotic-derived sulfenic acid metabolites in toxicology. Formation of sulfenic acid intermediates has been proposed in the metabolism of gitazones (Fig. 11) [34]. The secondary toxic effects found with some glitazones could be, at least in part, due to those electrophilic intermediates or to their S-oxide precursors and/or to their reaction products with GSH (Fig. 11), as all these species are electrophiles. Anyway, further studies are necessary to evaluate the possible roles of xenobiotic-derived sulfenic acid intermediates in toxicology.

References

[1] W.S. Allison, Acc. Chem. Res. 9 (1976) 293–299.
[2] A. Claiborne, H. Miller, D. Parsonage, R.P. Ross, FASEB J. 7 (15) (1993) 1483–1490.
[3] A. Claiborne, J.I. Yeh, T.C. Mallett, J. Luba, E.J. Crane 3rd, V. Charrier, D. Parsonage, Biochemistry 38 (47) (1999) 15407–15416.
[4] A. Claiborne, T.C. Mallett, J.I. Yeh, J. Luba, D. Parsonage, Adv. Protein. Chem. 58 (2001) 215–276.
[5] L.B. Poole, P.A. Karplus, A. Claiborne, Annu. Rev. Pharmacol. Toxicol. 44 (2004) 325–347.
[6] C. Jacob, I. Knight, P.G. Winyard, Biol. Chem. 387 (10–11) (2006) 1385–1397.
[7] L.V. Benitez, W.S. Allison, J. Biol. Chem. 249 (19) (1974) 6234–6243.
[8] L.B. Poole, B.B. Zeng, S.A. Knaggs, M. Yakubu, S.B. King, Bioconjugate Chem. 16 (6) (2005) 1624–1628.
[9] H.R. Ellis, L.B. Poole, Biochemistry 36 (48) (1997) 15013–15018.
[10] B. Testa, S.D. Kramer, Chem. Biodivers. 4 (3) (2007) 257–405.
[11] B. Testa, S.D. Kramer, Chem. Biodivers. 5 (11) (2008) 2171–2336.
[12] B. Testa, S.D. Kramer, Chem. Biodivers. 6 (5) (2009) 591–684.
[13] K.L. Taylor, D.M. Ziegler, Biochem. Pharmacol. 36 (1) (1987) 141–146.
[14] C.J. Decker, M.S. Rashed, T.A. Baillie, D. Maltby, M.A. Correia, Biochemistry 28 (12) (1989) 5128–5136.
[15] C.J. Decker, J.R. Cashman, K. Sugiyama, D. Maltby, M.A. Correia, Res. Toxicol. 4 (6) (1991) 669–677.

[16] D.C. Kossor, S. Kominami, S. Takemori, H.D. Colby, Mol. Pharmacol. 40 (2) (1991) 321–325.
[17] B.W. Hart, M.D. Faiman, Biochem. Pharmacol. 43 (3) (1992) 403–406.
[18] K. Yoneda, R. Iwamura, H. Kishi, Y. Mizukami, K. Mogami, S. Kobayashi, Br. J. Pharmacol. 142 (3) (2004) 551–557.
[19] J.M. Pereillo, M. Maftouh, A. Andrieu, M.F. Uzabiaga, O. Fedeli, P. Savi, M. Pascal, J.M. Herbert, J.P. Maffrand, C. Picard, Drug Metab. Dispos. 30 (11) (2002) 1288–1295.
[20] C. Gachet, Thromb. Haemost. 99 (3) (2008) 466–472.
[21] A. Tuong, A. Bouyssou, J. Paret, T.G. Cuong, Eur. J. Drug Metab. Pharmacokinet. 6 (2) (1981) 91–98.
[22] N.T. Ha-Duong, S. Dijols, A.C. Macherey, J.A. Goldstein, P.M. Dansette, D. Mansuy, Biochemistry 40 (40) (2001) 12112–12122.
[23] D.K. Dalvie, A.S. Kalgutkar, S.C. Khojasteh-Bakht, Chem. Res. Toxicol. 15 (3) (2002) 269–299.
[24] N.A. Farid, A. Kurihara, S.A. Wrighton, J. Clin. Pharmacol. 50 (2) (2010) 126–142.
[25] M. Kazui, Y. Nishiya, T. Ishizuka, K. Hagihara, N.A. Farid, O. Okazaki, T. Ikeda, A. Kurihara, Drug Metab. Dispos. 38 (1) (2010) 92–99.
[26] P.M. Dansette, J. Libraire, G. Bertho, D. Mansuy, Chem. Res. Toxicol. 22 (2) (2009) 369–373.
[27] A. Sugidachi, F. Asai, K. Yoneda, R. Iwamura, T. Ogawa, K. Otsuguro, H. Koike, Br. J. Pharmacol. 132 (1) (2001) 47–54.
[28] M. Hasegawa, A. Sugidachi, T. Ogawa, T. Isobe, J.A. Jakubowski, F. Asai, Thromb. Haemost. 94 (3) (2005) 593–598.
[29] E.T. Williams, K.O. Jones, G.D. Ponsler, S.M. Lowery, E.J. Perkins, S.A. Wrighton, K.J. Ruterbories, M. Kazui, N.A. Farid, Drug Metab. Dispos. 36 (7) (2008) 1227–1232.
[30] K. Hagihara, M. Kazui, A. Kurihara, H. Iwabuchi, M. Ishikawa, H. Kobayashi, N. Tanaka, O. Okazaki, N.A. Farid, T. Ikeda, Drug Metab. Dispos. 38 (6) (2010) 898–904.
[31] P.M. Dansette, S. Thebault, G. Bertho, D. Mansuy, Chem. Res. Toxicol. 23 (2010) 1268–1274.
[32] K. Goto, K. Shimada, M. Nagahama, R. Okazaki, T. Kawashima, Chem. Lett. 32 (11) (2003) 1080–1081.
[33] B.V. Karanam, C.E. Hop, D.Q. Liu, M. Wallace, D. Dean, H. Satoh, M. Komuro, K. Awano, S.H. Vincent, Drug Metab. Dispos. 32 (9) (2004) 1015–1022.
[34] V.B. Reddy, B.V. Karanam, W.L. Gruber, M.A. Wallace, S.H. Vincent, R.B. Franklin, T.A. Baillie, Chem. Res. Toxicol. 18 (5) (2005) 880–888.
[35] K. He, R.E. Talaat, W.F. Pool, M.D. Reily, J.E. Reed, A.J. Bridges, T.F. Woolf, Drug Metab. Dispos. 32 (6) (2004) 639–646.
[36] L. Jin, M.R. Davis, P. Hu, T.A. Baillie, Chem. Res. Toxicol. 7 (4) (1994) 526–533.
[37] P. Hu, L. Jin, T.A. Baillie, J. Pharmacol. Exp. Ther. 281 (2) (1997) 611–617.
[38] B.W. Hart, J.J. Yourick, M.D. Faiman, Alcohol 7 (2) (1990) 165–169.
[39] B.W. Hart, M.D. Faiman, Biochem. Pharmacol. 49 (2) (1995) 157–163.
[40] G.B. Quistad, S.E. Sparks, J.E. Casida, Life Sci. 55 (20) (1994) 1537–1544.
[41] R.E. Staub, S.E. Sparks, G.B. Quistad, J.E. Casida, Chem. Res. Toxicol. 8 (8) (1995) 1063–1069.
[42] N. Shirane, Z. Sui, J.A. Peterson, P.R. Ortiz de Montellano, Biochemistry 32 (49) (1993) 13732–13741.
[43] P.C. Feng, R.T. Solsten, Xenobiotica 21 (10) (1991) 1265–1271.
[44] R.E. Pearce, J.S. Leeder, G.L. Kearns, Drug Metab. Dispos. 34 (6) (2006) 1035–1040.
[45] F. Chanoine, C. Grenot, N. Sellier, W.E. Barrett, R.M. Thompson, A.F. Fentiman, J.R. Nixon, R. Goyer, J.L. Junien, Drug Metab. Dispos. 15 (6) (1987) 868–876.
[46] M.H. Potashman, M.E. Duggan, J. Med. Chem. 52 (5) (2009) 1231–1246.
[47] J.M. Shin, Y.M. Cho, G. Sachs, J. Am. Chem. Soc. 126 (25) (2004) 7800–7811.
[48] M.F. Tutunji, A.M. Qaisi, B.I. El-Eswed, L.F. Tutunji, J. Pharm. Sci. 96 (1) (2007) 196–208.
[49] D. Zhong, Z. Xie, X. Chen, J. Pharm. Pharmacol. 57 (3) (2005) 341–349.
[50] L. Jin, T.A. Baillie, Chem. Res. Toxicol. 10 (3) (1997) 318–327.
[51] R.T. Abraham, L.M. Benson, I. Jardine, J. Med. Chem. 26 (10) (1983) 1523–1526.
[52] I. Algaier, J.A. Jakubowski, F. Asai, I. von Kugelgen, J. Thromb. Haemost. 6 (11) (2008) 1908–1914.
[53] B.W. Hart, M.D. Faiman, Alcohol. Clin. Exp. Res. 18 (2) (1994) 340–345.
[54] H.D. Colby, J.P. O'Donnell, N.L. Flowers, D.C. Kossor, P.B. Johnson, M. Levitt, Toxicology 67 (2) (1991) 143–154.

Please cite this article in press as: D. Mansuy, P.M. Dansette, Arch. Biochem. Biophys. (2010), doi:10.1016/j.abb.2010.09.015