Mechanism of H₂S Formation from the Metabolism of Anetholedithiolethione and Anetholedithiolone by Rat Liver Microsomes

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ABSTRACT

The drug anetholedithiolethione (ADT) and its analogs have been extensively used as H₂S donors. However, the mechanism of H₂S formation from ADT under biologic conditions remains almost completely unknown. This article shows that only small amounts of H₂S are formed during incubation of ADT and of its metabolite anetholedithiolone (ADO) with rat liver cytosol or with rat liver microsomes (RLM) in the absence of NADPH, indicating that H₂S formation under these conditions is of hydrolytic origin only to a minor extent. By contrast, much greater amounts of H₂S are formed upon incubation of ADT and ADO with RLM in the presence of NADPH and dioxygen, with a concomitant formation of H₂S and *para*-methoxy-acetophenone (pMA). Moreover, H₂S and pMA formation under those conditions are greatly inhibited in the presence of *N*-benzyl-imidazole indicating the involvement of cytochrome P450-dependent monooxygenases. Mechanistic studies show the intermediate formation of the ADT-derived 1,2-dithiolium cation and

of the ADO sulfoxide during microsomal metabolism of ADT and ADO, respectively. This article proposes the first detailed mechanisms for the formation of H₂S from microsomal metabolism of ADT and ADO in agreement with those data and with previously published data on the metabolism of compounds involving a C=S bond. Finally, this article shows for the first time that ADO is a better H₂S donor than ADT under those conditions.

SIGNIFICANCE STATEMENT

Incubation of anetholedithiolethione (ADT) or its metabolite anetholedithiolone (ADO) in the presence of rat liver microsomes, NADPH, and O₂ leads to H₂S. This article shows for the first time that this H₂S formation involves several steps catalyzed by microsomal monooxygenases and that ADO is a better H₂S donor than ADT. We propose the first detailed mechanisms for the formation of H₂S from the microsomal metabolism of ADT and ADO.

Introduction

Anetholedithiolethione (ADT, formula in Fig. 1) is a drug that has been used for many years for its choleric and sialogogic properties (Christen, 1995; Hamada et al., 1999; Nagano and Takeyama, 2001). ADT and several dithiolethiones are also tested as cancer chemoprevention agents whose mechanisms of action involve activation of Nrf2 signaling and induction of phase 2 enzymes (Zhang and Munday, 2008; Ansari et al., 2018). ADT and its O-demethylated derivative dmADT were also extensively used as H₂S donors, and the coupling of dmADT with numerous anti-inflammatory drugs has led to a variety of compounds described for their H₂S-donor properties and therapeutic effects (Li et al., 2007; Chen et al., 2010; Lee et al., 2010; Sparatore et al., 2011; Kashfi and Olson, 2013; Couto et al., 2015; Szabo and Papapetropoulos, 2017; Ansari et al., 2018; Powell et al., 2018). However, the mechanism of H₂S formation from ADT, dmADT, and their derivatives under biologic conditions remains almost completely

unknown (Szabo and Papapetropoulos, 2017; Ansari et al., 2018; Powell et al., 2018).

We have recently reported that the oxidative metabolism of ADT by liver microsomes not only leads to dmADT but also to several products deriving from an *S*-oxidation of ADT, such as ADTSO, ADO, and *para*-methoxy-acetophenone (pMA) (Fig. 1) (Dulac et al., 2018). Under these conditions, the corresponding products deriving from dmADT, such as dmADTSO, dmADO, and *para*-hydroxy-acetophenone (pHA), are also formed (Fig. 1) (Dulac et al., 2018). Metabolism of ADO by the same microsomes leads to pMA, in which reaction the two sulfur atoms and one carbon atom of ADO are lost. Is there formation of H₂S during the cleavage of the heterocycle of ADT and ADO? In the study described in this article, we followed the formation of H₂S during the metabolism of ADT and ADO by rat liver microsomes (RLM) and found that ADO is a much better H₂S donor than ADT under these conditions. H₂S formation mainly occurs in the presence of liver microsomes and NADPH, the necessary cofactor of microsomal monooxygenases. Possible mechanisms for H₂S and pMA formation from ADT and ADO are proposed on the basis of these results.

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ABBREVIATIONS: ADO, anetholedithiolone, 5-(*p*-methoxyphenyl)-3H-1,2-dithiole-3-one; ADOSO, 5-(*p*-methoxyphenyl)-3H-1,2-dithiole-3-one sulfoxide; ADT, anetholedithiolethione, 5-(*p*-methoxyphenyl)-3H-1,2-dithiole-3-thione; ADTSO, 5-(*p*-methoxyphenyl)-3H-1,2-dithiole-3-thione sulfoxide; Bz-ImH, *N*-benzyl-imidazole; dmADT, O-demethylated derivative of ADT, 5-(*p*-hydroxyphenyl)-3H-1,2-dithiole-3-thione; DT, 5-(*p*-methoxyphenyl)-1,2-dithiolium hydrogensulfate; HLM, Human liver microsomes; HPLC-MS, high-performance liquid chromatography–mass spectrometry; HRMS, high-resolution mass spectrometry; β -NF, β -naphthoflavone; P450, cytochrome P450; PB, phenobarbital; pHA, *para*-hydroxy-acetophenone; pMA, *para*-methoxy-acetophenone; RLM, rat liver microsomes; UT-RLM, liver microsomes from untreated rats.

Fig. 1. Formula of the main metabolites formed during microsomal metabolism of ADT [from Dulac et al., 2018].

Materials and Methods

General Reagents and Synthesis of Authentic Samples. The commercial origins of ADT, pHA, pMA, NADPH, *N*-benzyl-imidazole (Bz-ImH), and the solvents used were indicated previously (Dulac et al., 2018). All authentic metabolites of ADT were prepared as described previously (Dulac et al., 2018) and displayed ^1H and ^{13}C NMR, UV-visible, and mass spectra in accordance with their structures. Nuclear magnetic resonance spectra were recorded on a Bruker 500 AV2 spectrometer. The mass spectra were recorded on an Exactive HRMS-instrument (Thermo, Les Ulis, France).

Synthesis of Dithiolium Hydrogensulfate. Dithiolium hydrogensulfate (DT) was prepared from ADT following a previously described protocol (Klingsberg, 1961). Five millimoles of ADT (1.2 g) were dissolved in 50 ml of acetone, and 2.8 g of 40% peracetic acid was added at 0°C . After 10 minutes, the precipitate was filtered, washed with acetone, and dried (1.4 g, 92%). ^1H NMR (D_2O): 10.04 (d, 1H, $J = 5.0$), 8.71 (d, 1H, $J = 5.0$), 8.06 (d, 2H, $J = 9.0$), 7.17 (d, 2H, $J = 9.0$), 3.97 (s, 3H). ^{13}C NMR (D_2O): 191.3, 171.2, 165.5, 134.8, 131.9, 123.4, 115.9, 56.0. HRMS: m/z value for $\text{C}_{10}\text{H}_9\text{OS}_2$: calc.: 209.0089, found: 209.0084. UV (MeOH) λ_{max} : 413 and 260 nm; UV (0.1 M HCl) λ_{max} : 404 and 290 nm.

Synthesis of ADOSO. ADOSO was prepared from ADO by oxidation with dimethyldioxirane (see formula in Fig. 3) following modifications of a previously described protocol (Tardif and Harp, 2000). A solution of ADO (11.2 mg, 0.05 mmol) in 1 ml acetone was cooled to -78°C and 1.4 ml of a solution of 0.04 M dimethyldioxirane in acetone [prepared as indicated in Adam et al. (1987)] was added. The temperature was allowed to rise to room temperature in 1 hour and the solvent was evaporated under vacuum. The crude product was found to be pure ADOSO. ^1H NMR (CDCl_3): 7.71 (d, 2H, $J = 9.0$), 7.02 (d, 2H, $J = 9.0$), 6.81 (s, 1H), 3.88 (s, 3H). ^{13}C NMR (CDCl_3): 190.2, 171.6, 163.6, 130.7, 121.8, 121.6, 115.0, 55.9. HRMS: m/z value for $\text{C}_{10}\text{H}_9\text{O}_3\text{S}_2$: calc.: 239.9915, found: 239.9908. UV (MeOH) λ_{max} : 306 nm.

Origin of Rat Liver Cytosols and Microsomes, and of Human Liver Microsomes. Rat liver microsomes and cytosols were prepared as previously reported (Kremers et al., 1981) from male Sprague-Dawley rats (Charles River, L'Arbresle, France) untreated (UT-RLM) or treated either with phenobarbital (PB) (50 $\text{mg}\cdot\text{kg}^{-1}$, in 0.9% NaCl, i.p. for 4 days) or β -naphthoflavone (β -NF) (50 $\text{mg}\cdot\text{kg}^{-1}$, in corn oil, i.p. for 4 days), two inducers of cytochromes P450 (P450) (Williams et al., 2005). Human liver microsomes were from Corning Inc. (Corning, Amsterdam, The Netherlands). Protein concentrations and P450 contents were determined as described previously (Bradford, 1976, and Omura and Sato, 1964, respectively).

Typical Microsomal Incubation Procedures with Measurement of H_2S Formation. Hydrogen sulfide formation was measured using the methylene blue method (Fogo and Popowsky, 1949; Li et al., 2007; Giustarini et al., 2014). In this assay, hydrogen sulfide is trapped by ZnSO_4 forming ZnS . Under acidic conditions, H_2S redissolves and reacts with *N,N*-dimethyl-*para*-phenylenediamine in an oxidative coupling reaction catalyzed by ferric chloride and forming methylene blue detected spectrophotometrically (λ_{max} : 670 nm). The usual assay mixture (final volume 300 μl) contained cytosolic (2.5 mg/ml) or microsomal (1.5 mg/ml , about 2.5 μM P450) proteins, 0.1 mM substrate, and 1 mM ZnSO_4 in 50 mM phosphate buffer, pH 7.4. The mixtures were preincubated for 2 minutes at 37°C , and the reactions were started by the addition of 1 mM NADPH. Usual incubations were carried out for 0–90 minutes at 37°C . The reactions were

quenched by the addition of 100 μl of 20 mM *N,N*-dimethyl-*para*-phenylenediamine sulfate (in 7.2 M HCl) and 100 μl of 30 mM FeCl_3 (in 1.2 M HCl). After 10 minutes at room temperature, the proteins were precipitated by centrifugation (13,000 rpm, 10 minutes), and absorbance at 690 nm of aliquots (200 μl) was determined using a 96-well microplate reader (PowerWaveXS; BioTek Instrument, Colmar, France). The H_2S concentrations were calculated against a calibration curve of Na_2S treated under identical conditions as the incubation mixtures. Incubations performed in the absence of O_2 were conducted as above, except that all components were prepared in potassium phosphate buffer previously bubbled with Argon for 30 minutes. Data are means $\pm$ S.D. from three to six experiments. Square deviation errors were determined by using the Microsoft Office Excel 2007 (STDEVPA function) software.

Analyses of Metabolites. Incubations (final volumes 200 μl) were performed under conditions identical to those described earlier, except that ZnSO_4 was omitted and reactions were quenched by the addition of 100 μl of cold methanol containing 50 μM 5-(*p*-chlorophenyl)-3H-1,2-dithiole-3-thione (internal standard). Treatment of the mixtures and their study by high-performance liquid chromatography–mass spectrometry (HPLC-MS) were done as described previously (Dulac et al., 2018).

Results and Discussion

Formation of H_2S upon Microsomal Metabolism of ADT and ADO. The formation of H_2S was followed by using the methylene blue method (Fogo and Popowsky, 1949; Giustarini et al., 2014) during the metabolism of ADT and ADO by liver cytosol or microsomes from PB-pretreated rats (see *Materials and Methods*). Table 1 shows that H_2S was formed upon incubation of 100 μM ADT with liver microsomes from PB-treated rats in the presence of NADPH in a yield of about 13% after 1 hour. Interestingly, metabolism of ADO led to a much better H_2S yield (about 40%) under identical conditions. It is noteworthy that only low amounts of H_2S were formed under the same conditions but in the absence of NADPH. Moreover, incubation of ADT or ADO with rat liver cytosol under the same conditions in the presence or in the absence of NADPH led to very low amounts of H_2S (Table 1). H_2S formation from metabolism of ADT or ADO with liver microsomes in the presence of NADPH greatly decreased in incubations performed under anaerobic conditions (Table 1). These data indicated that microsomal formation of H_2S from ADT or ADO mainly comes from an oxidation of these substrates catalyzed by microsomal monooxygenases. The inhibition of H_2S formation in aerobic microsomal incubations performed in the presence of Bz-ImH, a usual inhibitor of P450 (Testa and Jenner, 1981; Correia and Ortiz de Montellano, 2005) (Table 1), showed that the microsomal oxidations of ADT and ADO responsible for H_2S formation are mainly catalyzed by P450-dependent monooxygenases.

Similar results were obtained when either liver microsomes from untreated or β -NF-pretreated rats (UT-RLM or β -NF RLM) or human liver microsomes (HLM) were used, in the presence of NADPH, which led to yields of about 6% and 22% (UT-RLM), 12% and 42% (β -NF-RLM), or 9% and 17% (HLM) H_2S formation from ADT and ADO, respectively (data not shown).

When one simultaneously followed the formation of H_2S and the organic products derived from ADT or ADO in the presence of NADPH-supplemented microsomes, one always observed a concomitant formation of H_2S and pMA as a function of time. This is illustrated in Fig. 2, which shows the formation of H_2S and pMA during the metabolism of ADO by liver microsomes from PB-pretreated rats (35% yield on the basis of starting ADO, corresponding to about 14 total P450 turnovers). This is also illustrated in Table 1, which shows that similar amounts of H_2S and pMA are formed in all the conditions used to metabolize ADT and ADO. Altogether these data indicate that H_2S is formed during the metabolism of ADT and ADO to pMA. This is in agreement with the much lower yield of H_2S observed upon microsomal metabolism of

TABLE 1
Formation of H₂S upon metabolism of ADT and ADO by liver cytosol or microsomes from PB-pretreated rats

Conditions	H ₂ S ^a	pMA ^a	Conditions	H ₂ S ^a	pMA ^a
ADT + NADPH	1.0 ± 0.3	0.1 ± 0.1	ADO + NADPH	1.0 ± 0.5	0.2 ± 0.1
ADT + Cytosol	2.0 ± 1.0	0.1 ± 0.1	ADO + Cytosol	2.0 ± 1.5	3.5 ± 1.5
ADT + Cytosol + NADPH	2.8 ± 1.0	0.1 ± 0.1	ADO + Cytosol + NADPH	3.0 ± 1.8	3.7 ± 1.5
ADT + Microsomes	2.7 ± 0.7	0.8 ± 0.5	ADO + Microsomes	3.0 ± 1.5	0.5 ± 0.2
ADT + Microsomes + NADPH	12.7 ± 2.0	20.5 ± 5.0	ADO + Microsomes + NADPH	40.0 ± 5.0	41.0 ± 6.0
ADT + Microsomes + NADPH – O ₂	3.8 ± 1.5	5.0 ± 2.5	ADO + Microsomes + NADPH – O ₂	5.0 ± 2.0	8.2 ± 2.5
ADT + Microsomes + NADPH + Bz-ImH ^b	5.2 ± 1.5	4.5 ± 2.0	ADO + Microsomes + NADPH + Bz-ImH ^b	6.5 ± 1.5	5.5 ± 2.0

^aH₂S and pMA yields measured after 1-h incubation of ADT or ADO (100 μM) in phosphate buffer, pH 7.4, in the presence of cytosol (1.5 mg/ml of proteins) or microsomes (2.5 μM P450) from PB-pretreated rats, as described in *Materials and Methods*. Data are percentage with starting ADT or ADO as a basis and are means ± S.D. from three to six experiments.

^b1 mM *N*-benzyl-imidazole was added in the incubation.

ADT that was found to lead to dmADT as a major product (about 65% yield) and only to lower amounts of pMA under similar conditions (Dulac et al., 2018). The concomitant formation of H₂S and pMA from microsomal oxidation of ADT and ADO is in agreement with the fact that both H₂S (this work) and pMA (Dulac et al., 2018) are mainly derived from reactions catalyzed by microsomal monooxygenases.

Possible Mechanism of H₂S Formation during Microsomal Metabolism of ADO. The results just described indicate that H₂S formation from microsomal metabolism of ADO is catalyzed by monooxygenases. A possible mechanism for this reaction is shown in Fig. 3. It would involve an *S*-oxidation of ADO followed by the opening of the S–S bond of the intermediate (ADOSO) by H₂O to give intermediate **1**. The hydrolysis of the HSC=O function of **1** should lead to H₂S and compound **2**. Decarboxylation of **2** would lead to **3**, the disulfide of *para*-methoxy-thioacetophenone. Further monooxygenation of **3** should lead to pMA, as previously found in microsomal oxidation of compounds involving a C=S bond (Cashman and Hanzlik, 1982; Hanzlik and Cashman, 1983; Vannelli et al., 2002; Testa and Krämer, 2007).

The sulfoxide intermediate proposed in Fig. 3, ADOSO, was synthesized following a previously described method (Tardif and Harp, 2000) (see *Materials and Methods*) and found to be stable in the buffer used for microsomal incubations (Table 2). In the presence of RLM without NADPH, it led to H₂S formation in a 33% yield after a 1-hour incubation. This result is in agreement with the mechanism shown in Fig. 3, in which a simple hydrolysis of ADOSO leads to the formation of H₂S. However, hydrolysis of the O=S–S bond of ADOSO appears to be catalyzed by liver microsomal proteins as it does not occur in buffer alone (Table 2). In the presence of liver microsomes and NADPH, the H₂S yield increased up to about 61% (Table 2). This could be a result of an *S*-oxidation of the S=O group of ADOSO catalyzed by microsomal monooxygenases followed by the hydrolytic opening of the resulting O₂S–S bond and hydrolysis of the HSC=O bond.

Possible Mechanism of H₂S Formation during Microsomal Metabolism of ADT. The possible formation of intermediates in the metabolism of ADT to H₂S and pMA was evaluated from a high-performance liquid chromatography–high-resolution mass spectrometry (HRMS) study of incubates of ADT with liver microsomes from PB-pretreated rats in the presence of NADPH. During the metabolism of ADT under these conditions, one could detect, in addition to the previously reported metabolites dmADT, ADTSO, ADO, dmADO, and pMA (Dulac et al., 2018), small amounts of the 1,2-dithiolium cation DT (Fig. 4), derived from a loss of the sulfur atom of the ADT C=S group. DT was characterized by its high-resolution mass spectrometry molecular peak at *m/z* = 209.0084 (theoretical *m/z* value for C₁₀H₉OS₂ = 209.0089). The formation of the hydrogensulfate of such a 1,2-dithiolium ion has been reported in the oxidation of 4-phenyl-1,2-dithiol-3-thione, an ADT analog, by three equivalents of peracetic acid

(Klingsberg, 1961). Oxidation of ADT by peracetic acid under such conditions led us to DT hydrogensulfate, the cation characterized by ¹H NMR and HRMS (see *Materials and Methods*). The HRMS spectrum of this authentic compound was identical to that of DT detected during ADT microsomal oxidation. Incubation of 100 μM DT hydrogensulfate with liver microsomes from PB-pretreated rats in the presence of NADPH led to H₂S (38% yield, Table 2) and pMA (30% yield, data not shown), respectively, after 1 hour.

Thus, ADO and DT are formed during microsomal metabolism of ADT and lead to H₂S and pMA when incubated with RLM in the presence of NADPH. Possible mechanisms for their formation upon microsomal oxidation of ADT are shown in Fig. 4. They would involve a common intermediate, previously found in microsomal *S*-oxidation of compounds involving a C=S function (Cashman and Hanzlik, 1982; Hanzlik and Cashman, 1983; Vannelli et al., 2002; Testa and Krämer, 2007), that results from two successive *S*-oxidations of the C=S function (Cashman and Hanzlik, 1982; Hanzlik and Cashman, 1983; Vannelli et al., 2002; Testa and Krämer, 2007), intermediate **4** in the case of ADT. Monooxygenation of **4** leading to **5** could be followed by an attack of H₂O either on the carbon atom of **5** leading to **6** (Fig. 4, Pathway A) or on the highly oxidized sulfur atom of **5** leading to **8** (Fig. 4, Pathway B). In pathway A, further *S*-oxidation of intermediate **6** would lead to intermediate **7**. The loss of a hydrogensulfate moiety from **7** would then lead to ADO (Fig. 4). Possible microsomal mechanisms for the formation of H₂S from ADO have been discussed in the previous

Fig. 2. Representative kinetics of formation of H₂S and pMA upon oxidation of 100 μM ADO by liver microsomes from PB-pretreated rats (2.5 μM P450) in the presence of 1 mM NADPH. Incubations (500 μl) were performed and stopped at the times indicated in *Materials and Methods*. H₂S amounts (●) were measured by the methylene blue assay from 300 μl aliquots, whereas the pMA levels (○) were obtained from 200 μl aliquots analyzed by HPLC with UV-visible detection.

absence of NADPH. This indicates that H₂S formation under these conditions is of hydrolytic origin only to a minor extent. By contrast, much greater amounts of H₂S are formed upon incubation of ADT and ADO with RLM only in the presence of NADPH and dioxygen, the two necessary reactants of microsomal monooxygenases. Moreover, H₂S formation under those conditions is greatly inhibited in the presence of Bz-ImH, indicating that this formation is mainly catalyzed by P450-dependent monooxygenases. This article also shows for the first time that ADO is a much better H₂S donor than ADT under these conditions. It also proposes for the first time detailed mechanisms for the formation of H₂S from the metabolism of ADT and ADO that are in agreement with the previously described data on the metabolism of these compounds (Dulac et al., 2018) and the mechanisms previously proposed for the metabolic oxidation of compounds involving a C=S bond (Cashman and Hanzlik, 1982; Hanzlik and Cashman, 1983; Vannelli et al., 2002; Testa and Krämer, 2007).

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Authorship Contributions

Participated in research design: Dansette, Boucher, Mansuy.

Conducted experiments: Dulac, Nagarathinam, Boucher.

Contributed new reagents or analytic tools: Dulac, Boucher, Dansette.

Performed data analysis: Dulac, Boucher, Dansette, Mansuy.

Wrote or contributed to the writing of the manuscript: Mansuy, Boucher, Dansette.

References

- Adam W, Chan YY, Cremer D, Gauss J, Scheutzw D, and Schindler M (1987) Spectral and chemical properties of dimethyldioxirane as determined by experiment and ab initio calculations. *J Org Chem* **62**:2800–2803.
- Ansari MI, Khan MM, Saquib M, Khatoun S, and Hussain MK (2018) Dithiolethiones: a privileged pharmacophore for anticancer therapy and chemoprevention. *Future Med Chem* **10**:1241–1260.
- Böttcher B and Lüttringhaus A (1945) Über trithione. *Justus Ann Chem* **557**:89–107.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254.
- Cashman JR and Hanzlik RP (1982) Oxidation and other reactions of thiobenzamide derivatives of relevance to their hepatotoxicity. *J Org Chem* **47**:4645–4650.
- Chen P, Luo Y, Hai L, Qian S, and Wu Y (2010) Design, synthesis, and pharmacological evaluation of the aqueous prodrugs of desmethyl anethole trithione with hepatoprotective activity. *Eur J Med Chem* **45**:3005–3010.
- Christen MO (1995) Anethole dithiolethione: biochemical considerations. *Methods Enzymol* **252**:316–323.
- Correia MA and Ortiz de Montellano PR (2005) Inhibition of cytochromes P450 enzymes, in *Cytochrome P450, Structure, Mechanism, and Biochemistry*, 3rd ed (Ortiz de Montellano PR ed) pp 247–322, Kluwer Academic/Plenum Publishers, New York.
- Couto M, de Ovale S, Cabrera M, Cerecetto H, and González M (2015) Searching phase II enzymes inducers, from Michael acceptor-[1,2]dithiolethione hybrids, as cancer chemopreventive agents. *Future Med Chem* **7**:857–871.
- Dulac M, Sassi A, Nagarathinam C, Christen MO, Dansette PM, Mansuy D, and Boucher JL (2018) Metabolism of anethole dithiolethione by rat and human liver microsomes: formation of various products deriving from its O-demethylation and S-oxidation. Involvement of cytochromes P450 and flavin monooxygenases in these pathways. *Drug Metab Dispos* **46**:1390–1395.
- Fogo JK and Popowsky M (1949) Spectrophotometric determination of hydrogen sulfide. *Anal Chem* **21**:732–734.
- Giustarini D, Fanti P, Sparatore A, Matteucci E, and Rossi R (2014) Anethole dithiolethione lowers the homocysteine and raises the glutathione levels in solid tissues and plasma of rats: a novel non-vitamin homocysteine-lowering agent. *Biochem Pharmacol* **89**:246–254.
- Hamada T, Nakane T, Kimura T, Arisawa K, Yoneda K, Yamamoto T, and Osaki T (1999) Treatment of xerostomia with the bile secretion-stimulating drug anethole trithione: a clinical trial. *Am J Med Sci* **318**:146–151.
- Hanzlik RP and Cashman JR (1983) Microsomal metabolism of thiobenzamide and thiobenzamide S-oxide. *Drug Metab Dispos* **11**:201–205.
- Kashfi K and Olson KR (2013) Biology and therapeutic potential of hydrogen sulfide and hydrogen sulfide-releasing chimeras. *Biochem Pharmacol* **85**:689–703.
- Klingsberg E (1961) The 1,2-Dithiolium cation. A new pseudoaromatic system. I Preparation and properties of 3-phenyl- and 4-phenyl-1,2-dithiolium salts. *J Am Chem Soc* **83**:2934–2937.
- Kremers P, Beaune P, Cresteil T, de Graeve J, Columelli S, Leroux JP, and Gielen JE (1981) Cytochrome P-450 monooxygenase activities in human and rat liver microsomes. *Eur J Biochem* **118**:599–606.
- Lee M, Tazzari V, Giustarini D, Rossi R, Sparatore A, Del Soldato P, McGeer E, and McGeer PL (2010) Effects of hydrogen sulfide-releasing L-DOPA derivatives on glial activation: potential for treating Parkinson disease. *J Biol Chem* **285**:17318–17328.
- Li L, Rossoni G, Sparatore A, Lee LC, Del Soldato P, and Moore PK (2007) Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative. *Free Radic Biol Med* **42**:706–719.
- Nagano T and Takeyama M (2001) Enhancement of salivary secretion and neuropeptide (substance P, alpha-calcitonin gene-related peptide) levels in saliva by chronic anethole trithione treatment. *J Pharm Pharmacol* **53**:1697–1702.
- Omura T and Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**:2370–2378.
- Powell CR, Dillon KM, and Matson JB (2018) A review of hydrogen sulfide (H₂S) donors: chemistry and potential therapeutic applications. *Biochem Pharmacol* **149**:110–123.
- Sparatore A, Santus G, Giustarini D, Rossi R, and Del Soldato P (2011) Therapeutic potential of new hydrogen sulfide-releasing hybrids. *Expert Rev Clin Pharmacol* **4**:109–121.
- Szabo C and Papapetropoulos A (2017) International Union of Basic and Clinical Pharmacology. Pharmacological modulation of H₂S levels: H₂S donors and H₂S biosynthesis inhibitors. *Pharmacol Rev* **69**:497–564.
- Testa B and Jenner P (1981) Inhibitors of cytochrome P-450s and their mechanism of action. *Drug Metab Rev* **12**:1–117.
- Testa B and Krämer SD (2007) The biochemistry of drug metabolism—an introduction: Part 2. Redox reactions and their enzymes. *Chem Biodivers* **4**:257–405.
- Tardif S and Harp D (2000) Chemoselective oxidation of 5-phenyl-1,2-dithiole-3-substituted derivatives to their corresponding 5-phenyl-1,2-dithiole-S-oxides. *Sulfur Lett* **23**:169–184.
- Vannelli TA, Dykman A, and Ortiz de Montellano PR (2002) The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. *J Biol Chem* **277**:12824–12829.
- Williams SN, Dunham E, and Bradfield CA (2005) Induction of cytochrome P450 enzymes, in *Cytochrome P450, Structure, Mechanism, and Biochemistry*, 3rd ed (Ortiz de Montellano PR ed) pp 323–346, Kluwer Academic/Plenum Publishers, New York.
- Zhang Y and Munday R (2008) Dithiolethiones for cancer chemoprevention: where do we stand? *Mol Cancer Ther* **7**:3470–3479.

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