First Evidence that Cytochrome P450 catalyzes both S-oxidation and Epoxidation of Thiophene Derivatives

Patrick M. Dansette*, Gildas Bertho and Daniel Mansuy

Université Paris Descartes, UFR Biomédicale, CNRS UMR 8601, 45 Rue des Saints-Pères, 75270 Paris Cedex 06, France.

Tel : +33(0)142862191

Fax : +33(0)142868387

e.mail : Patrick.Dansette@univ-paris5.fr

ABSTRACT

Oxidation of 2-phenylthiophene (**2PT**) by rat liver microsomes, in the presence of NADPH and glutathione (GSH), led to three kinds of metabolites whose structures were established by ^{1}H NMR and mass spectrometry. The first ones were **2PT**-S-oxide dimers formed by Diels-Alder type dimerization of **2PT**-S-oxide, while the second ones were GSH adducts deriving from the 1,4-Michaël-type addition of GSH to **2PT**-S-oxide. The third metabolites were GSH adducts resulting from a nucleophilic attack of GSH to the 4,5-epoxide of **2PT**. Oxidation of **2PT** by recombinant, human cytochrome P4501A1, in the presence of NADPH and GSH, also led to these three kinds of metabolites. These results provide the first evidence that cytochrome P450 may catalyze the oxidation of thiophene compounds with the simultaneous formation of two reactive intermediates, a thiophene-S-oxide and a thiophene epoxide.

Key-words : 2-phenylthiophene, thiophene-S-oxide dimers, thiophene-S-oxide-glutathione adduct, thiophene epoxide-glutathione adduct, CYP1A1, rat liver microsomes, arene oxide.

Arene oxides derived from cytochrome P450-dependent oxidation of aromatic compounds play a central role in the oxidative metabolism of these compounds [1]. Two electrophilic, unstable intermediates are *a priori* possible in the oxidative metabolism of thiophene compounds in mammals, the thiophene-S-oxides and thiophene epoxides [2, 3]. So far, several publications have provided evidence for the formation of thiophene-S-oxides as major intermediates [2, 4-7], whereas no direct evidence for the formation of thiophene epoxides intermediates has been reported. Thus, in rats, the metabolites of thiophene itself found *in vitro* and *in vivo* are sulfoxide dimers resulting from the Diels-Alder type dimerization of thiophene-S-oxide [6] and glutathione adducts coming from the Michaël-type addition of glutathione to thiophene-S-oxide [2, 6] (Figure 1). The oxidative metabolism of a 3-aroylthiophene analog of tienilic acid also leads to metabolites deriving from reactions of glutathione with its S-oxide intermediate [5]. *In vivo* and *in vitro* oxidation of several 2-aroylthiophenes mainly leads to metabolites resulting from their hydroxylation at position 5 [8-14]. These 5-hydroxy metabolites could result from an isomerization of either a thiophene-S-oxide intermediate, or a 4,5-epoxide intermediate [15].

We have recently studied the oxidation of 2-phenylthiophene **(2PT)** either by rat liver microsomes or by recombinant cytochrome P4501A1 (CYP1A1), and observed the formation of metabolites deriving both from 2-phenylthiophene-S-oxide and from 2-phenylthiophene epoxide. These results provide the first evidence that cytochrome P450-dependent oxidation of thiophenes may occur with the simultaneous formation of S-oxide and epoxide intermediates.

MATERIALS AND METHODS

All reagents were of the highest quality commercially available. **2PT** was purchased from Aldrich (L'Isle d'Abeau Chenes, France).

Tritiation of 2PT. Tritiation at position 5 of **2PT** was performed by taking advantage of the previously reported easy exchange of thiophene hydrogens at C_2 and C_5 under acidic conditions [2, 6]. A mixture of 20 mg **2PT**, 200 μl trifluoroacetic acid, and 30 μl tritiated water (16 μCi/μmole) in 2 ml CH_2Cl_2 was stirred for 24h at 40°C. After dilution with 10 ml CH_2Cl_2, the solution was washed eight times with H_2O, dried on $MgSO_4$ and evaporated to dryness, leading to 20 mg of [5-^{3}H]-**2PT** (0.7 μCi/μmole).

Microsomal incubations and HPLC analysis. Liver microsomes from rats pretreated with β-naphthoflavone (50 mg/kg/day i.p. for 3 days) were prepared as described previously [16]; they contained 1.3 nmol P450/mg protein. Microsomes from yeast expressing human CYP1A1 and yeast NADPH-cytochrome P450 reductase were obtained according to previously described techniques [17]; they contained 0.18 nmol CYP1A1/mg protein.

For analytical measurements (HPLC and HPLC-MS analyses), incubations were done in 0.1M phosphate buffer pH 7.4 containing 1mM EDTA, **2PT** (0.1 to 1.5mM), microsomes (protein amounts corresponding to 0.3μM P450) and a NADPH-generating system (1mM NADP, 10mM glucose-6-phosphate and 2 unit/ml glucose-6-phosphate dehydrogenase), at 28°C (yeast microsomes) or 37°C (rat microsomes), for times between 1 and 30 min. Reactions were stopped with addition of 0.5 volume of CH_3CN containing 4% acetic acid. After centrifugation at 10 000g for 10 min, the supernatant was analyzed by HPLC using a Hypersil MOS column (5 μm, 250 x 4.6 mm) and a 20 min linear gradient from A = 0.1M ammonium acetate pH 4.6 to B = CH_3CN, CH_3OH, H_2O (7 : 2 : 1) for 20 min at a flow of 1 ml/min. For radioactivity analyses, 0.5 min fractions were collected in 3 ml polyethylene tubes and counted after addition of 2 ml picofluor 40 in a scintillation counter (Perkin-Elmer, Saint Quentin en Yvelines, France).

HPLC-MS studies : Incubations were performed as described above in 1 ml total volume ; the supernatant was loaded onto OASIS columns (Waters, Saint Quentin en Yvelines, France), washed with 1 ml H_2O and eluted with 1 ml CH_3OH. After concentration to 0.2 ml, 20 μl of the final solution was analyzed with a HPLC Surveyor coupled to an ion-trap mass spectrometer (Thermofinnigan LCQ Advantage, Orsay, France), using a Kromasil C18 column (3.5 μm, 100 mm x 2.1 mm) and a linear gradient of A/B mixture from 0% B to 80% B in 20 min at 200 μl/min (A = 0.1 % formic acid in H_2O, B = CH_3CN containing 0.1% formic acid). MS parameters were 275°C for capillary temperature and 5 KeV for capillary voltage.

^{1}H NMR studies : ^{1}H NMR spectra were recorded on Bruker WM250 and AM 500 spectrometers. Chemical shifts (δ) are given in ppm relative to $(CH_3)_4Si$ and J in Hz. Abbreviations used for singlet, doublet, doublet of doublets, broad singlet and massif are s, d, dd, bs and m, respectively. Cys, Gly and Glu are used for cystein, glycine and glutamate respectively. For preparation of larger amounts of metabolites necessary for ^{1}H NMR analysis, incubations of 80 ml rat liver microsomal suspensions containing 98 nmol P450 and

500 µM **2PT**, in the presence of the NAPDH-generating system, were performed for 1h at 37°C. After addition of 1.6 ml acetic acid and centrifugation, the supernatant was loaded on SepPak C18 cartridges (20 ml/cartridge). Each cartridge was washed with 3 ml H_2O and eluted with 2 ml CH_3OH. The metabolites were separated by HPLC (see above), lyophilyzed, dissolved in 0.5 ml D_2O or $CDCl_3$ and studied by ^{1}H NMR spectroscopy.

Metabolites 2PTSOD. Their ^{1}H NMR spectra (in $CDCl_3$) were found to be identical to those of previously described 2PT-S-oxide dimers [18]. The major isomer was 2,4-diphenyl-*cis*-3a, 4, 7, 7a-tetrahydro-*cis*-4,7-epithio-1-benzothiophene, *trans*-1, *syn*-8-dioxide (Fig.2), whereas the minor one was the corresponding *cis*-1, *syn*-8 dioxide isomer.

Metabolite 2PTGA. ESIMS (ElectroSpray Ionization Mass Spectrometry), m/z = 484 (M + H) ; MS-MS on ion 484, m/z = 466(-H_2O, 15%), 436(-SO, 50%), 409(10%), 355(100%), 337(20%), 191(20%), 177(15%) ; UV spectrum : λmax = 246 nm. ^{1}H NMR (250 MHz, D_2O) δ = 7.3-7.5(m, 5H, phenyl), 6.34(m, 1H, H_3), 6.30(d, 5.7 Hz, 1H, H_4), 5.91(bs, 1H, H_5), 5.23(bd, 2.5 Hz, 1H, H_2), 4.66(m, 1H, αCys), 3.85(m, 3H, αGlu + αGly), 3.2-3.5(m, 2H, βCys), 2.62(m, 2H, γGlu), 2.2(m, 2H, βGlu). From the shape of the signal observed for H_3 and the β protons of the Glu residue, **2PTGA** appeared to be a mixture of at least two diastereoisomers.

Metabolite 2PTGB. ESIMS, m/z = 484 (M + H); MS-MS on ion 484, m/z = 466(-H_2O, 100%), 337(25%), 308(60%). UV spectrum : λmax = 286 nm. The ^{1}H NMR spectrum corresponds to a mixture of two diastereoisomers A and B in a 3 : 2 ratio. ^{1}H NMR(500 MHz, D_2O) : δ = 7.65(d, 7.7 Hz, 2H, orthophenyl), 7.50(m, 3H, phenyl), 6.19 (A) and 6.16 (B) (d, 3.5 Hz, 1H, H_3), 5.71 (B) and 5.70 (A) (s, 1H, H_5), 4.70(m, 1H, αCys), 4.41 (B) and 4.39 (A) (d, 3.5 Hz, 1H, H_4), 3.76(m, 3H, αGlu and αGly), 3.2 (A) and 3.15 (B) (dd, 4.5 and 14 Hz, 1H, βCys), 3.02 (A) and 2.9 (B) (dd, J = 9 and 14 Hz, 1H, βCys), 2.5(m, 2H, γGlu), 2.1(m, 2H, βGlu). The NOESY (Nuclear Overhauser Effect SpectroscopY) spectrum of **2PTGB** clearly showed an interaction between the vinyl proton and the ortho-phenyl protons, which is only compatible with the presence of this proton on C_3.

The use of the HMQC (Heteronuclear Multiple Quantum Correlation), HMBC (Heteronuclear Multiple Band Correlation) and HSQC (Heteronuclear Single Quantum Correlation) two-dimension NMR techniques [19] allowed us to determine the ^{13}C chemical shifts of some carbons of **2PTGB** : δ = 173(COGlu), 169(COGly), 168(COCys), 128, 126.7, 123.9 and 123.8 (phenyl), 112.4 (C_3), 84.5 (C_5), 58.3 (C_4), 52 (CαCys), 51 (CαGlu), 41

(CαGly), 28.6 (Cγ Glu) and 23.8 (CβGlu). The signals corresponding to C_3, C_4 and C_5 also showed the presence of two diastereoisomers.

RESULTS

2-Phenylthiophene (**2PT**), partially tritiated at position 5, [5-^{3}H]-2-phenylthiophene, was incubated with liver microsomes from β-naphthoflavone-pretreated rats in the presence of NADPH. Analysis of the incubation mixture by HPLC-MS showed the major formation of two 2-phenylthiophene-S-oxide dimers, **2PTSOD**, that should result from the Diels-Alder type dimerization of 2-phenylthiophene-S-oxide. These **2PTSOD** metabolites were fully characterized by their mass and ^{1}H NMR spectra, by comparison with those of authentic samples prepared by oxidation of **2PT** with *meta*-chloroperbenzoic acid in the presence of BF_3 etherate, a method previously described for the synthesis of other thiophene-S-oxide dimers by dimerization of thiophene-S-oxide intermediates generated *in situ* [20]. The spectral data of the two **2PTSOD** metabolites, that were formed in a 5 : 1 molar ratio, were found to be in complete agreement with those of two previously described stereoisomers of 2PT-S-oxide dimers [18]. The stereochemistry of the major isomer is shown in Fig.2 ; it only differs from that of the minor isomer by the configuration of the S_1 atom (*trans*-1-oxide instead of *cis*-1-oxide). It is noteworthy that the stereochemistry observed for the **2PTSOD** metabolites completely corresponded to those described previously for other thiophene-S-oxide dimers ; it should derive from the stereoelectronic control of the Diels-Alder type dimerization [18, 20].

The HPLC-MS analysis of the reaction mixture also showed the formation of trace amounts of **2PTT** (2-phenylthiolene thiolactone) that could derive from a 5-hydroxylation of **2PT**. This metabolite was found to be identical (HPLC retention time and mass spectrum) to an authentic sample of **2PTT** that was synthesized according to a previously described procedure [21]. It is an unstable compound that rapidly leads to "thio-indigo-like" condensed, colored products [22, 23]. Accordingly, after incubation with **2PT** and NADPH, liver microsomes became highly colored (from blue to brown depending upon the experimental conditions). Because of these further complex reactions of **2PTT**, the only way to evaluate the extent of **2PTT** formation was to measure the amount of tritiated water released upon microsomal oxidation of [5-^{3}H]-2-phenylthiophene. Incubation (30 min) of [5-^{3}H]-

6

2PT(300μM) with rat liver microsomes in the presence of a NADPH generating system led to a 35% conversion of [5-^{3}H]-**2PT** into **2PTSOD** and to the release of ^{3}H$_2$O(30%) under the used conditions (Table 1). Identical incubations but in the absence of NADPH failed to lead to any ^{3}H$_2$O release and to any formation of **2PTSOD**.

Microsomal incubations under identical conditions but in the presence of 5 mM glutathione (GSH) led to a dramatic decrease of the formation of **2PTSOD** and tritiated water (Table 1), and of the blue coloration of the microsomes. HPLC-MS analysis of the reaction mixture showed the formation of two new metabolites with respective yields of 20 and 30% based on starting [5-^{3}H]-**2PT** (Table 1). Analysis of these metabolites by mass spectrometry and ^{1}H NMR spectroscopy using COSY (COrrelated SpectroscopY), NOESY, HMQC, HBQC and HSQC techniques [19] showed that the new metabolites were glutathione adducts. The mass spectra of these two metabolites all exhibited a molecular ion at M + H = 484 corresponding to **2PT** + O + GSH + H. The ^{1}H NMR spectrum of the most polar metabolite, **2PTGA**, showed the presence of four protons that are characteristic of a 2,5-dihydrothiophene ring (see Materials and Methods). This spectrum was highly similar to those previously described for compounds derived from the Michaël-type addition of thiols to thiophene-S-oxides [2, 5, 6, 24]. It was in complete agreement with the structure of **2PTGA** shown in Fig.2, that results from the 1,4-addition of glutathione to **2PT**-S-oxide. From its 1H NMR spectrum, **2PTGA** appeared to be a mixture of at least two major stereoisomers ; however this spectrum did not allow one to determine the relative configurations of the C$_2$, S$_1$ and C$_5$ centers. The less polar glutathione adducts, **2PTGB**, were a 3:2 mixture of two diastereoisomers whose ^{1}H NMR spectrum showed the presence of only three protons for the dihydrothiophene ring. Two dimension-^{1}H NMR analysis was in agreement with a structure resulting from the addition of glutathione to the 4,5-epoxide of **2PT**, with an hydroxy group on carbon 5 and the GS group on carbon 4 (Fig.2). This regiochemistry is in agreement with the ^{13}C chemical shifts found for C$_5$ and C$_4$ (84.5 and 58.3 ppm respectively), as a function of literature data for carbons bearing a SR and an OH substituent (for C$_5$), and a SR and a vinyl substituent (for C$_4$) respectively [25]. The *trans* relative position of the OH and SG subtituents is in agreement with the low coupling constant observed between H$_4$ and H$_5$ (< 1 Hz). A *cis* relative position should have led to a larger coupling constant, as *cis*-4,5-diols of 4,5-dihydrothiophenes exhibit a coupling constant between the H$_4$ and H$_5$ protons larger than

5 Hz [18]. These data suggest that **2PTGB** is a 3 : 2 mixture of diastereoisomers of 2-phenyl-*trans*-5-hydroxy-4-S-glutathionyl-4,5-dihydrothiophene.

The structures indicated (Fig.2) for these two different kinds of glutathione adducts, **2PTGA** and **2PTGB**, were in agreement with their different behaviours in MS-MS spectrometry (**2PTGA** looses 48 corresponding to SO, whereas **2PTGB** mainly looses 18 corresponding to H_2O), and in an acidic medium (**2PTGA** is stable for more than one hour at pH1, whereas **2PTGB** undergoes a very fast dehydration to 2-phenyl S-glutathionyl thiophene (M + H = 466) under identical conditions).

These data show that microsomal oxidation of **2PT** occurred with simultaneous formation of a thiophene-S-oxide and an arene oxide on the thiophene ring. Since liver microsomes contained several cytochrome P450 isozymes, it was not possible to conclude from these results whether these two intermediates were formed by the same cytochrome P450 or by two different isozymes. In order to solve this problem, we then studied the oxidation of **2PT** by recombinant human CYP1A1. A similar study of incubations of **2PT** with microsomes of yeast co-expressing human CYP1A1 and yeast NADPH cytochrome P450 reductase, in the presence of NADPH and glutathione, led to results similar to those obtained with rat liver microsomes, with the formation of the same metabolites (Table 1). The less efficient trapping of the S-oxide and epoxide intermediates by GSH in the case of recombinant CYP1A1, relative to what it is in the case of liver microsomes, could be due to a more efficient catalysis of these GSH reactions by glutathione transferase(s) in rat liver microsomes.

DISCUSSION

The aforementioned results provide a supplementary evidence for the intermediate formation of thiophene-S-oxides in the microsomal oxidation of thiophene derivatives. As previously described in the case of thiophene itself [6] and of the thiophene-containing drug, ticlopidine [26], **2PT** is oxidized by liver microsomes, with formation of thiophene-S-oxide dimers resulting from Diels-Alder dimerization of the corresponding thiophene-S-oxide intermediate. As in the case of thiophene [2, 6] and a 3-aroylthiophene [4, 5], oxidation of **2PT** by rat liver microsomes in the presence of a thiol nucleophile (GSH here) led to the formation of metabolites deriving from the addition of the thiol to the intermediate thiophene-

S-oxide. The ^{1}H NMR study of these metabolites showed that the major isomer formed results from a 1,4-Michaël-type addition of the thiol to the thiophene-S-oxide (as depicted in Fig.2). This regiochemistry of the thiol addition was previously reported for other chemically-prepared thiophene-S-oxides [24].

More interestingly, the aforementioned results show for the first time that microsomal oxidation of a thiophene derivative not only leads to the intermediate formation of an S-oxide but also to that of an arene-oxide of the thiophene ring. The precise stereochemistry of the two diastereoisomers of the **2PTGB** metabolite is not definitely established, however a detailed analysis of their ^{1}H NMR spectrum strongly suggests that they derive from a nucleophilic attack of GSH to the 4,5-oxide of **2PT**, at position 4 (Fig.2). Thus, the formation of **2PTSOD** and **2PTGA**, as well as of **2PTGB**, in recombinant CYP1A1-dependent reactions clearly shows that a cytochrome P450 may oxidize a thiophene compound with the simultaneous formation of two intermediates, a thiophene-S-oxide and a thiophene epoxide. Further studies are necessary to know whether this phenomenon is general ; recent results obtained in this laboratory indicate that microsomal oxidation of 3-phenylthiophene also leads to metabolites derived from the corresponding S-oxide and epoxide intermediates (P. Dansette and D. Mansuy, in preparation). Another question that requires further studies is concerned with the mechanism of formation of **2PTT**, the 5-hydroxylated metabolite of **2PT**. As previously discussed for the 5-hydroxylated products of several 2-aroylthiophenes [15], these metabolites could derive from an isomerization of either a thiophene epoxide or a thiophene S-oxide.

9

References

[1] D. Jerina, and J. Daly, Arene oxides: a new aspect of drug metabolism, Science 185 (1974) 573-582.

[2] P. M. Dansette, D. C. Thang, H. el Amri, and D. Mansuy, Evidence for thiophene-S-oxide as a primary reactive metabolite of thiophene in vivo: formation of a dihydrothiophene sulfoxide mercapturic acid, Biochem Biophys Res Commun 186 (1992) 1624-1630.

[3] D. K. Dalvie, A. S. Kalgutkar, S. C. Khojasteh-Bakht, R. S. Obach, and J. P. O'Donnell, Biotransformation reactions of five-membered aromatic heterocyclic rings, Chem Res Toxicol 15 (2002) 269-299.

[4] D. Mansuy, P. Valadon, I. Erdelmeier, P. Lopez-Garcia, C. Amar, J. P. Girault, and P. M. Dansette, Thiophene S-Oxides As New Reactive Metabolites - Formation by Cytochrome-P450 Dependent Oxidation and Reaction with Nucleophiles, J Am Chem Soc 113 (1991) 7825-7826.

[5] P. Valadon, P. M. Dansette, J. P. Girault, C. Amar, and D. Mansuy, Thiophene sulfoxides as reactive metabolites: formation upon microsomal oxidation of a 3-aroylthiophene and fate in the presence of nucleophiles in vitro and in vivo, Chem Res Toxicol 9 (1996) 1403-1413.

[6] A. Treiber, P. M. Dansette, H. ElAmri, J. P. Girault, D. Ginderow, J. P. Mornon, and D. Mansuy, Chemical and biological oxidation of thiophene: Preparation and complete characterization of thiophene S-oxide dimers and evidence for thiophene S-oxide as an intermediate in thiophene metabolism in vivo and in vitro, J Am Chem Soc 119 (1997) 1565-1571.

[7] N. T. Ha-Duong, S. Dijols, A. C. Macherey, J. A. Goldstein, P. M. Dansette, and D. Mansuy, Ticlopidine as a selective mechanism-based inhibitor of human cytochrome P450 2C19, Biochemistry 40 (2001) 12112-12122.

[8] D. Mansuy, P. M. Dansette, C. Foures, M. Jaouen, G. Moinet, and N. Bayer, Metabolic hydroxylation of the thiophene ring: isolation of 5-hydroxy-tienilic acid as the major urinary metabolite of tienilic acid in man and rat, Biochem Pharmacol 33 (1984) 1429-1435.

[9] E. Neau, P. M. Dansette, V. Andronik, and D. Mansuy, Hydroxylation of the thiophene ring by hepatic monooxygenases. Evidence for 5-hydroxylation of 2-aroylthiophenes as a general metabolic pathway using a simple UV-visible assay, Biochem Pharmacol 39 (1990) 1101-1107.

[10] Y. Mori, Y. Sakai, N. Kuroda, F. Yokoya, K. Toyoshi, M. Horie, and S. Baba, Further structural analysis of urinary metabolites of suprofen in the rat, Drug Metab Dispos 12 (1984) 767-771.

[11] S. Ichihara, Y. Tsuyuki, H. Tomisawa, H. Fukazawa, N. Nakayama, M. Tateishi, and R. Joly, Metabolism of tenoxicam in rats, Xenobiotica 14 (1984) 727-739.

[12] M. J. Lynch, F. R. Mosher, W. R. Levesque, and T. J. Newby, The in vitro and in vivo metabolism of morantel in cattle and toxicology species, Drug Metab Rev 18 (1987) 253-288.

[13] T. Nishikawa, O. Nagata, K. Tanbo, T. Yamada, Y. Takahara, H. Kato, and Y. Yamamoto, Absorption, excretion and metabolism of tiquizium bromide in dogs, and relationship between pharmacological effect and plasma levels of unchanged drug, Xenobiotica 15 (1985) 1053-1060.

[14] H. G. Fouda, M. J. Avery, D. Dalvie, F. C. Falkner, L. S. Melvin, and R. A. Ronfeld, Disposition and metabolism of tenidap in the rat, Drug Metab Dispos 25 (1997) 140-148.

[15] M. Belghazi, P. Jean, S. Poli, J. M. Schmitter, D. Mansuy, and P. M. Dansette, Use of isotopes and LC-MS-ESI-TOF for mechanistic studies of tienilic acid metabolic activation, Adv Exp Med Biol 500 (2001) 139-144.

[16] P. M. Dansette, C. Amar, C. Smith, C. Pons, and D. Mansuy, Oxidative activation of the thiophene ring by hepatic enzymes. Hydroxylation and formation of electrophilic metabolites during metabolism of tienilic acid and its isomer by rat liver microsomes, Biochem Pharmacol 39 (1990) 911-918.

[17] J. C. Gautier, P. Urban, P. Beaune, and D. Pompon, Engineered Yeast Cells as Model to Study Coupling Between Human Xenobiotic Metabolizing Enzymes - Simulation of the two first Steps of Benzo<a>pyrene Activation, Eur J Biochem 211 (1993) 63-72.

[18] D. R. Boyd, N. D. Sharma, N. Gunaratne, S. A. Haughey, M. A. Kennedy, J. F. Malone, C. C. R. Allen, and H. Dalton, Dioxygenase-catalysed oxidation of monosubstituted thiophenes: sulfoxidation versus dihydrodiol formation, Org Biomol Chem 1 (2003) 984-994.

[19] W.R. Croasmun, and R.M.K. Carlson, Two-Dimensional NMR Spectroscopy: Applications for chemists and biochemists, ed., VCH Publishers, New York 1994.

[20] Y. Q. Li, T. Thiemann, T. Sawada, S. Mataka, and M. Tashiro, Lewis acid catalysis in the oxidative cycloaddition of thiophenes, J Org Chem 62 (1997) 7926-7936.

[21] B. A. Hornfeldt, Unsaturated γ-thiolactones. IV. Preparations, tautomeric structures, and tautomeric equilibria of some 5-substituted thiolen-2-ones, Arkiv foer Kemi 22 (1964) 211-235.

[22] A. Kosak, R. F. Palchak, W. A. Steele, and C. M. Selwitz, The synthesis and properties of the 5-phenylthiophene-2-and 3-ols, J Am Chem Soc 76 (1954) 4450-4454. .

[23] N. Heindel, J. Minatelli, and D. Harris, Salicylidene-thiolactone rearrangement. A direct synthesis of 4H-2-arylthieno[3,2-c][1]benzopyran-4-ones, J Org Chem 42 (1977) 1465-1466.

[24] J. Nakayama, Studies on pi-face selective additions with 3,4-di-tert-butylthiophene 1-oxide and 1-imide, J Synth Org Chem Japan 61 (2003) 1106-1115;.

[25] A. Furst, and E. Pretsch, A computer program for the prediction of 13-C-NMR chemical shifts of organic compounds, Analytica Chimica Acta 229 (1990) 17-25.

[26] N. T. Ha-Duong, S. Dijols, A. C. Macherey, P. M. Dansette, and D. Mansuy, Inhibition by ticlopidine and its derivatives of human liver cytochrome p450. Mechanism-based inactivation of CYP 2C19 by ticlopidine, Adv Exp Med Biol 500 (2001) 145-148.

Table 1 : Relative amounts of metabolites formed upon oxidation of 2PT by β-naphthoflavone-pretreated rat liver microsomes or yeast-expressed human CYP1A1, in the presence (or absence) of GSH[a].

Metabolites	Yields (%)[b]		CYP1A1
	rat liver microsomes		
	- GSH	+ GSH	+ GSH
2PTSOD	35	12	31
2PTT (from ^3H_2O release)	30	3	4
2PTGA	0	20	6
2PTGB	0	30	3

(a) Conditions : 30 min incubation of 300 μM [5-^{3}H]-**2PT** with either β-naphthoflavone-pretreated rat liver microsomes (0.3 μM P450) or microsomes of yeast expressing human CYP1A1 (0.28 μM) and yeast NADPH cytochrome P450 reductase, in the presence of a NAPH-generating system (1mM NADPH). + GSH : presence of 5 mM GSH in the incubation mixture. (b) Yields relative to starting [5-^{3}H]-**2PT** were calculated from the radioactivity of each metabolite separated by HPLC (see Materials and Methods).

Fig.1 : *In vivo* and *in vitro* **metabolism of thiophene in rats (from ref.2 and 6).**

The glutathione (GSH) adduct shown in this figure is only an intermediate ; the final metabolite found in urine is the corresponding mercapturate.

Fig.2 : Oxidative metabolism of 2PT by rat liver microsomes or recombinant human CYP 1A1 in the presence of GSH.

The stereochemistry shown for **2PTSOD** is that of the major stereoisomer. The relative configuration of the C_2, C_5 and S_1 centers of **2PTGA** is not known. In the case of **2PTGB**, only the structure of one of the two main diastereoisomers formed is shown. The second diastereoisomer should derive from an inversion of configuration of C_4 and C_5. In order to facilitate the understanding of the involved reactions, the numbering of the thiophene atoms of **2PT** was also used for the **2PTGA** and **2PTGB** metabolites. The official nomenclature for **2PTGA** and **2PTGB** would have required a different numbering, with the phenyl substituent being at position 5.

Fig.1

Fig.2