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A NEW THIOPHENE METABOLITE: NADPH, O₂ AND THIOL DEPENDENT FORMATION OF A THIOPHENE HYDRATE FROM (S)-MTPPA BY CYP2C9

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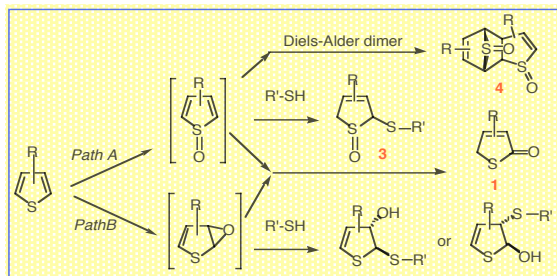
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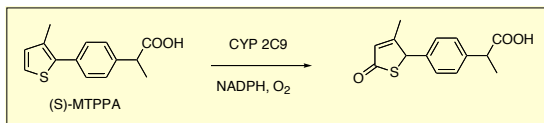
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Thiophenes are metabolized in rat or human liver microsomes to hydroxy-thiophenes¹ and thiophenes sulfoxides which may dimerize or be trapped as nucleophile-adducts². The major metabolites of thiophene are *in vivo* the N-2-acetylcytosteine S-substituted 2,5-dihydrothiophene-1-oxide³ but *in vitro* the S-oxide dimers⁴. A number of 2-aryl-thiophenes are however metabolized to the 5-hydroxy derivatives¹. We have shown recently that 2-phenyl and 3-phenyl thiophenes are metabolized to thiolenones (5-hydroxy-thiophene isomers) and thiophene-sulfoxide dimers, and in presence of glutathione to two type of adducts corresponding to addition of glutathione to **both the intermediate thiophene epoxide(s) and thiophene S-oxide**.



(S)-MTPPA ((S)-2-[4-(3-methyl-2-thienyl)phenyl]propionic acid) is a NSAID recently introduced. It is metabolized by CYP 2C9 into a 5-hydroxythiophene metabolite⁶ existing mostly as a thiolen-2-one tautomer.



AIM

The aim of this study was to investigate if a potential epoxide intermediate could be trapped as a glutathione adduct as in the case of 2-phenylthiophene and 3-phenylthiophene.

RESULTS

Incubation with recombinant CYP2C9 gave the thiolenone. Adding progressive amount (0.1 – 10 mM) GSH to the incubation decreased the formation of the 5-hydroxythiophene metabolite and a new more polar metabolite appeared (see Figure 1 and 2).

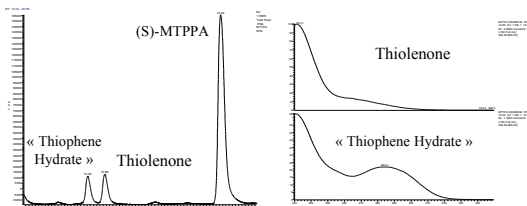
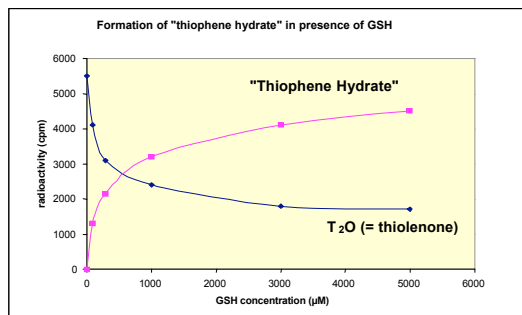
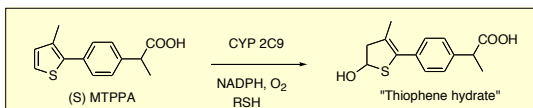


Figure 1: HPLC trace and UV spectra of an incubation with CYP2C9, NADPH and GSH (5 mM); the thiophene hydrate elute at 15.3 min, the thiolenone at 15.9 min and the MTPPA at 19 min.



To our **surprise** this new product did not contain GSH.

Use of 5-Tritium labeled (S)-MTPPA showed that contrary to the thiolenone, the new metabolite was radioactive and retained the tritium. The thiolenone has no conjugation with the phenyl group, and its UV spectrum absorbs only at 240 nm. The new metabolite has a band at 280 nm and is probably conjugated. Establishment of its structure by multidimensional NMR (see table 1 and spectrum 1 and 2) and MS shows that it is a **5-hydroxy-4,5-dihydro-thiophene derivative, formally a "thiophene hydrate"**.



At neutral pH this new metabolite is relatively **stable** (T1/2 # 3 days). It is acid labile (20 mM TFA, (T1/2 < 15 min) and dehydrates to the parent thiophene. GSH can be replaced by other thiols (N-Acetylcysteine or mercaptoethanol).

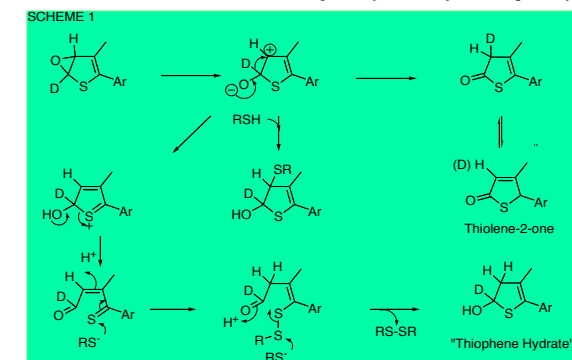
	H (C)	H	C
2	5;52, s	-----	129.3
3	-----	-----	??
4a	6.20, s	2.91, d J _{4a,4b} =17.7 Hz	55
4b	-----	3.34, dd J _{4a,4b} =17.7 Hz, J _{4,5} = 5.9 Hz	
5	-----	J _{4,5} = 5.9 Hz	81
1'	-----	-----	145.4
2'	7.27, d J _{2',3'} = 7.9 Hz	7.38, d J _{2',3'} = 7.9 Hz	130.1
3'	7.35, d J _{2',3'} = 7.9 Hz	7.42, d J _{2',3'} = 7.9 Hz	131.4
4'	-----	-----	130.7
1''	-----	-----	185.7
2''	3.75	3.69, q J _{2'',3''} = 7.1 Hz	50.7
3''	1.48, d J _{2'',3''} = 7.1 Hz	1.43, d J _{2'',3''} = 7.1 Hz	21
3-Me	1.96, s	1.90, s	17.6

Table 1: Selected NMR values for the 2 metabolites of (S)-MTPPA, the thiolen-2-one, and the new "thiophene hydrate".

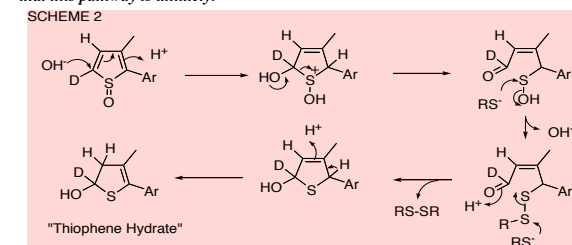
Potential mechanism of formation

Two mechanistic pathways are shown on Scheme 1 and Scheme 2:

Scheme 1 shows a thiophene 4,5-epoxide intermediate which would be common for formation of the thiolen-2-one and of the thiophene hydrate. **We favor this pathway.**



Scheme 2 on the other hand uses a thiophene 1-oxide as intermediate. Although on paper the mechanism is plausible, since thiols are better nucleophile than water it is astonishing that no thiophene 1-oxide-thiol adduct could be trapped⁸, contrary to the case of 2-phenylthiophene, a simpler analog of MTPPA. **Thus we believe that this pathway is unlikely.**



Finally this mechanism is not specific of (S)-MTPPA, since recent work shows that 2-phenylthiophene and 2-thienyl-4-benzoic acid also can be metabolized in presence of GSH or mercaptoethanol to low amount of the corresponding "thiophene hydrate"

CONCLUSION : In presence of thiols, NADPH and O₂ CYP 2C9 metabolizes (S)-MTPPA into a new type of metabolite, a « thiophene hydrate ». This corresponds to an oxidation plus a reduction of the thiophene ring.

Methods: (S)-MTPPA was labeled in CH₂Cl₂ by exchange with tritiated TFA obtained by mixing TFA anhydride with tritiated water and labeled with deuterated TFA yielding 5-deutero (S)-MTPPA thiophene. Metabolic oxidation of (S)-MTPPA (0.2 mM) was performed by recombinant CYP2C9 expressed in yeast in presence of a NADPH generating system. Incubations in presence glutathione (0.1 - 6 mM), mercaptoethanol (10 mM) or N-acetylcystein (6 mM) were compared. Scale up to 40 ml allowed isolation of enough metabolite for characterisation by NMR and mass spectroscopy. HPLC were on Hyperstil MOS (5 µ, 250 x 4.6 mm). NMR: 250 and 500 MHz (Bruker), HPLC-ESI MS: Surveyor and LCQ Advantage (ThermoFinnigan). Sample purification: SepPak C18 or Oasis(Waters). **Acknowledgments:** We thank Isabelle Vanhee (summer student) for her skilful help.

References : 1) Neau E. et al. Biochemical Pharmacol. 1990; 39: 1101-07; 2) Mansuy D. et al. J. Am.Chem. Soc. 1991; 113: 7825-26; 3) Dansette P.M. et al. Biochem. Biophys. Res. Commun. 1992; 186: 1624-30; 4) Treiber A. J. Am. Chem. Soc. 1997; 119: 1565-71. 5) Treiber et al. J. Org Chem 2003, 68, 20060 and 67, 7261-66. 6) Dalvie, D.K et al. Chem. Res. Toxicol.; 2002; 15: 269-299. 7) Li et al. J. Org Chem. 1997, 62, 7926-30. 8) Otani T. et al. Chem. Letters, 2000, p 744-745.