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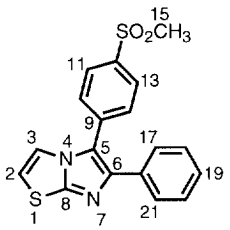
CHARACTERIZATION OF THE IN VITRO OXIDATIVE METABOLITES OF THE COX-2 SELECTIVE INHIBITOR L-766,112

Laird A. Trimble,* Nathalie Chauret, José M. Silva, Deborah A. Nicoll-Griffith, Chun-Sing Li, and James A. Yergey

Merck Frosst Centre for Therapeutic Research
CP 1005, Pointe Claire-Dorval, Québec, H9R 4P8, Canada

Abstract: L-766,112 (**1**), a potent and selective inhibitor of human COX-2, was incubated with rat and rhesus monkey hepatic microsomal fractions under oxidative conditions to generate a metabolite identified as the thiophene *S*-oxide. This oxide was trapped with glutathione to give a conjugate in which GSH added to the 3 position. The metabolites, also identified in rat hepatocyte incubations, were characterized by UV, NMR, and CF-LSIMS. Copyright © 1996 Elsevier Science Ltd

In the previous paper,[1] the discovery of L-766,112 (**1**), a potent and selective inhibitor of cyclooxygenase-2 (COX-2), was described. This compound was shown to be active in both in vitro and in vivo assays and its pharmacokinetic profile was presented. Recent literature suggests that the thiophene ring system is subject to oxidative metabolism to give the chemically reactive thiophene *S*-oxide.[2] This oxide is a strong electrophile and can covalently bind to hepatic proteins inducing toxic effects. This metabolic pathway has been carefully studied in the case of the diuretic drug, tienilic acid.[3] Although **1** does not contain a thiophene ring, the imidazo[2.1-b]thiazole heterocycle may undergo similar metabolic processes. This paper describes our efforts to characterize the oxidative metabolism of L-766,112.



1 L-766,112

Metabolic studies on L-766,112 were carried out with control rat and rhesus monkey hepatic microsomal fractions under oxidative conditions similar to those reported previously.[4] The parent compound was recovered to the extent of 75% and 65% after 1 h in rat and rhesus monkey microsome incubations, respectively. A major metabolite (M1) was detected in both species (Figure 1). Addition of glutathione (GSH) to the incubations under oxidative conditions[5] resulted in less metabolite M1 and the formation of a new very polar metabolite (M2). The UV spectrum of M1, M2, and the parent compound were all different, suggesting that metabolism had occurred on the chromophore. A large scale incubation of rhesus monkey microsomes under oxidative conditions[4] in the absence and presence of glutathione was performed in order to make sufficient amounts of the metabolites for NMR characterization.

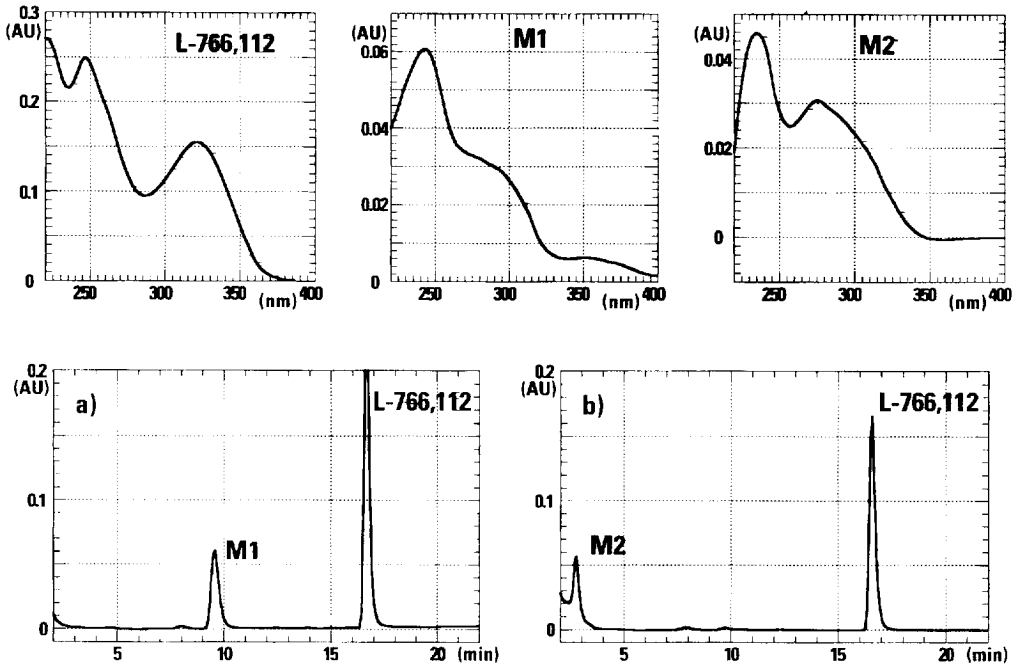
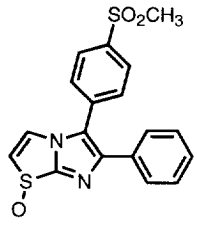


Figure 1. HPLC traces from Rhesus monkey microsomal incubations of L-766,112 in (a) the absence and (b) the presence of GSH as well as the UV spectra of the parent and two metabolites, M1 and M2. HPLC was performed using a Zorbax C_{18} column (150 × 4.6 mm) and a gradient mobile phase where eluent A was 20 mM NH_4OAc (pH 7) and eluent B was methanol. A linear gradient from 60% A to 30% A with a flow rate of 1 mL/min was applied over a 22 min period.

The capillary HPLC/CF-LSIMS[6] results suggested the addition of one oxygen (MH^+ = 371 Da with facile loss of 16 Da) to the core of **1**. Facile loss of 16 Da (O) suggested oxidation on one of the heteroatoms, or the production of a 2,3-epoxide. Unfortunately, no further fragmentaion was observed making it difficult to differentiate between these possibilities using MS. ^1H and ^{13}C NMR studies were carried out in order to resolve this problem. 1-D proton spectra and 2-D DQF-COSY[7] experiments on M1 showed that all of the protons in the parent compound **1** were also found in the metabolite (see Table 1). The chemical shifts of M1 were, for the most part, unchanged relative to **1** indicating that the site of oxidation must be at one of the heteroatoms. The ^{13}C chemical shifts of M1 were assigned using a combination of HMQC[8] and HMBC[8] techniques and are also given in Table 1. Comparison of the carbon chemical shifts of M1 and **1** show that the greatest changes occur in the thiazole ring suggesting that the metabolite is a *S*-oxide. It is unlikely that oxidation had occurred at the 4 or 7 positions since, in the case of the former, the resulting loss of aromaticity would result in large changes in chemical shift in both rings and, in the case of the latter, the largest chemical shift changes would have occurred in the imidizole and not the thiazole ring. These results suggest that M1 is the *S*-oxide thiazole **2**.



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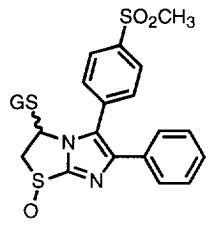

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Table 1. ^{1}H and ^{13}C chemical shifts for L-766,112 (**1**), its oxide **2**, and the glutathione adduct **3**. In the case of **3**, the chemical shifts for the glutathione moiety are not given. All chemical shifts are relative to the residual DMSO solvent signal which was set to 2.49 and 39.5 ppm for ^{1}H and ^{13}C spectra, respectively.

	1		**2**		**3**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	7.39	113.91	7.20	124.01	4.56, 3.77	63.02
3	7.90	118.32	7.90	129.49	6.24	60.33
5		120.71		124.53		125.67
6		143.26		143.28		145.15
8		149.16		151.33		152.11
9		134.13		132.99		133.96
10,14	7.74	129.07	7.77	130.22	7.84	130.21
11,13	8.01	127.32	8.06	127.14	8.06	127.28
12		139.45		140.88		140.79
15	3.30	43.15	3.32	42.91	3.31	42.93
16		133.47		132.99		132.73
17,21	7.52	127.13	7.47	126.64	7.43	126.41
18,20	7.35	128.18	7.37	128.29	7.33	128.05
19	7.30	127.13	7.33	127.51	7.28	127.22

Treatment of the microsomal incubation mixture with glutathione produced a new metabolite M2 that appeared to be a glutathione conjugate of the *S*-oxide **2**. Capillary HPLC/CF-LSIMS examination of the incubation mixture confirmed the formation of a glutathione adduct of the monooxidized product (MH^+ = 678 Da). Examination of the proton spectra showed that the metabolite was a 3:1 mixture of two very closely related compounds. The 1-D ^{1}H and 2-D DQF-COSY experiments indicated that, with the exception of the 2 and 3 protons, all of the signals found in the parent were present in the major component of the mixture (Table 1). In addition, the spin systems for the glutathione moiety were identified as was a new aliphatic AMX spin system consisting of coupled methine and methylene groups. An NOE was observed between the new methine signal and protons 10/14 in the 2-D NOESY[7] experiment indicating that the likely site of addition of GSH is at the 3 position to give the glutathione adduct **3**. This structure was confirmed upon analysis of the HMQC and HMBC spectra which showed three bond correlations between the H-3 methine and the cysteine β protons. The ^{1}H and ^{13}C chemical shifts of the major isomer are given in Table 1.

Addition of GSH to **2** creates a new chiral center and since the GSH is itself chiral, there is a possibility of generating two diastereomers. It is clear from the 1-D proton and the 2-D DQF-COSY spectra that the two components in the metabolite mixture contain identical proton spins systems. Unfortunately, the carbon assignments for the minor component could not be obtained because there was insufficient material to give adequate S/N. However, the similarity of the proton spectra suggest that the two components are indeed the expected diastereomers formed by the addition of GSH to metabolite **2**.

Since metabolic studies carried out with hepatocytes are thought to better reflect in vivo metabolism in that both Phase I and Phase II metabolites are produced, such experiments were carried out. L-766,112 was found to be extensively metabolised when it was incubated with control rat hepatocytes under typical conditions (2×10^6 cells/mL, 37 °C, 3 h).[9,10] Only 10% of the parent compound was present at the end of the incubation and two metabolites having the same retention time and UV spectra as M1 and M2 were detected. These results suggest that the same two metabolites that were identified in the microsomal incubations are produced by hepatocytes. The degree to which the *S*-oxide was covalently bound to cellular protein could not be determined without the use of a radioactive form of **1**.

Previous work has shown that L-766,112 has a high degree of selectivity and activity for human COX-2. However, a major metabolite of this compound is the *S*-oxide which is a potent Michael acceptor which could lead to covalent protein binding, resulting in toxic effects. This suggests that **1** is not an optimal drug candidate and that analogues of this compound without the thiazole ring should be explored to reduce this potential metabolic problem while maintaining the desired selectivity and potency.

References

1. See preceding paper.
2. Beaune, P.; Dansette, P. M.; Mansuy, D.; Kiffel, L.; Finck, M.; Amar, C.; Leroux, J. P.; Homberg, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 551; Mitchell, J. R.; Nelson, W. L.; Potter, W. Z.; Sasame, H. A.; Jollow, J. A. *J. Pharmacol. Exp. Ther.* **1976**, *119*, 41.
3. López-Garcia, M. P.; Dansette, P. M.; Mansuy, D. *Biochemistry* **1994**, *33*, 166, Mansuy, D.; Valadon, P.; Erdelmeier, I.; López-Garcia, P.; Amar, C.; Girault, J. P.; Dansette, P. M. *J. Am. Chem. Soc.* **1991**, *113*, 7825.
4. Nicoll-Griffith, D. A.; Yergey, J. A.; Trimble, L. A.; Williams, H.; Rasori, R.; Zamboni, R. *Drug Metabolism Disp.* **1992**, *20*, 383.
5. Nicoll-Griffith, D. A.; Chauret, N.; Yergey, J. A.; Trimble, L. A.; Favreau, L.; Zamboni, R.; Grossman, S. G.; Drey, J.; Herold, E. *Drug Metabolism Disp.* **1993**, *21*, 861.
6. Li, C.; Chauret, N.; Ducharme, Y.; Trimble, L. A.; Nicoll-Griffith, D. A.; Yergey, J. A. *Anal. Chem.* **1995**, *67*, 2931.
7. Kessler, H.; Gehrke, M.; Griesinger, C. *Angew. Chem.* **1988**, *27*, 490.
8. Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287.
9. Moldeus, P.; Hogberg, J.; Orrenius, S. *Methods Enzymol.* **1978**, *52*, 60.
10. Silva, J. M.; Nicoll-Griffith, D. A.; Yergey, J. A. The biotransformation of Verlukast isolated rat hepatocytes. *6th North American ISSX Meeting*. **1994**, Raleigh, NC.

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