METABOLISM OF A CYTOSOLIC PHOSPHOLIPASE $A_2\alpha$ INHIBITOR WITH AN ACTIVATED KETO GROUP BY RAT LIVER MICROSOMES

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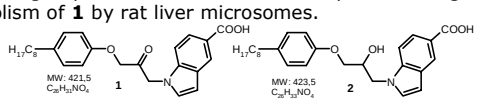
Introduction:

Inhibitors of cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$) are assumed to provide a novel therapeutic with application in many inflammatory diseases. Recently we have found that 1-[3-(4-octylphenoxy)-2-oxypropyl]indole-5-carboxylic acid (**1**) is a potent inhibitor of cPLA$_2\alpha$ with an activated ketone moiety as pharmacophore (serine trap) [1]. Since it is known that this functional group shows metabolic instability we investigated the metabolism of **1** by rat liver microsomes.



It is important to evaluate the metabolic stability of drug candidates toward CYP-mediated metabolism, and to use it as parameter in identifying and optimizing drug candidates for further development [2].

Though inter-species differences and variations of CYP isoforms are known [3] metabolism by rat liver microsomes give an idea about stability and potential bioavailibility of substances.

Microsomal Incubation:

The liver microsomes of male Sprague-Dawley rats were preparated adapted to published procedures [4,5].

Microsomal incubations were carried out in 0.1 M potassium phosphate buffer pH 7.4 at 37 °C in a shaking water bath and contained rat microsomes (2.25 [mg/ml] protein / Bradford / BSA standards), 3 mM $MgCl_2$ and 1.9 mM NADPH. The final addition of the analyt (20 µM) initiated the metabolisation which was carried out for 30 min (2h for LC/MS-analysis). Reference incubations were performed without NADPH. Liquid-liquid-extraction of the assay with diethylether was accomplished after addition of internal standard, reaction termination by acetonitrile and acidification. The separated organic layer was concentrated under a stream of nitrogen and the residue was dissolved in mobile phase.

LC-Analysis:

As the activated ketone moiety of **1** exhibits hydrate formation in aqueous solutions, besides the peak of **1** a peak for its hydrate form could be detected during RP-chromatography (see figure 6). Hence, a normal-phase chromatography with isohexane/THF was employed.

A 50 µl volume of the resolved assay residue was injected onto Lichrospher 100-5 CN (250 x 4 mm, pre column 4x4 mm) column (Merck, Darmstadt) at a flow rate of 0.75 ml/min, column oven temperature of 25 °C and a UV detection wavelength of 240 nm. The mobile phase consisted of isohexane / THF 92:8 (0.1% TFA) (A) and isohexane / THF 50:50 (0.1% TFA) (B). The following gradient was applied (A%): 0 min: 90, 15 min: 20, 18 min: 20, 20 min: 90, 27 min: 90.

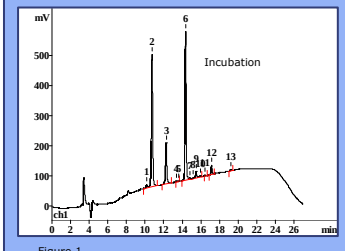


Figure 1

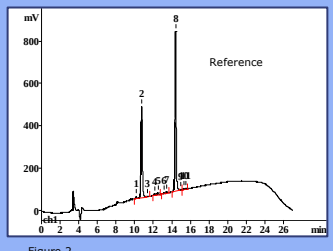


Figure 2

Results:

The chromatogram of the reference (figure 2) displays the internal standard (peak no. 2: 10.8 min) and the analyt peak of **1** (peak no. 8: 14.4 min) as main peaks. The incubation (figure 1) resulted in the formation of metabolites (peak no. 3: 12.3 min, no. 9: 15.5 min, no. 12: 17.2 min). Peak 3 was the main metabolite with about 18% of the peak area ratio of **1** in the reference assay. Approximately 69% of **1** were not metabolised within 30 min.
After spiking the incubation probe with substance **2**, it could be assumed that this compound was the main metabolite.

LC/MS^n-Analysis:

LC/MS^n was employed to verify the alcohol **2** as the main metabolite.

Since LC/MS investigations with normal-phase chromatography were not successful, a RP-chromatographic method had to be developed.

A 100 µl volume of the dissolved assay residue was injected onto Kromasil 100-5 C18 (60 x 2 mm, inletfilter 2 µm) column (CS, Langerwehe) at a flow rate of 0.4 ml/min and a UV detection wavelength of 235 nm. The mobile phase consisted of 20mM ammonium formiate / acetonitrile 80:20 (A) and 20mM ammonium formiate / acetonitrile 15:85 (B). The following gradient was applied (A%): 0 min: 55, 10 min: 0, 11 min: 0, 12 min: 55, 15 min: 55

Two minutes after injection, the MS-detector (LCQ® Thermo-Finnigan) was activated and structure determination was performed using ESI-MS^n in negative mode.

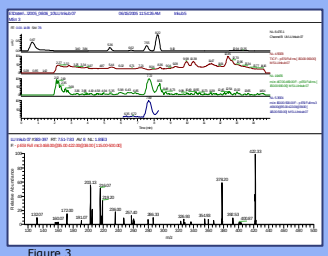


Figure 3

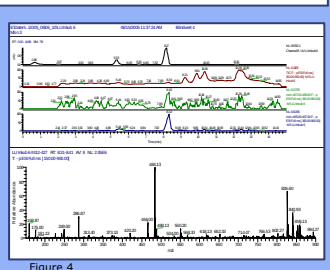


Figure 4

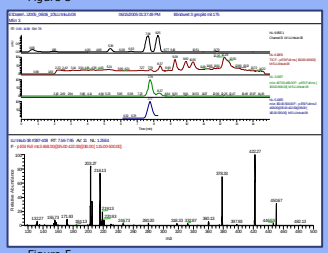


Figure 5

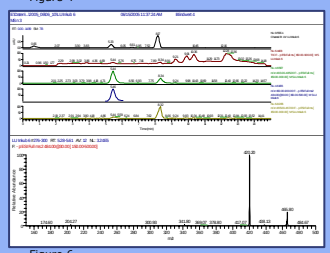


Figure 6

LC/MS^n results:

The alcohol **2** was confirmed as the main metabolite with rat liver microsomes. Representive ESI-MS and MS^3 data of the incubation assay are shown in figure 3, which reveal a formiate adduct of **2** at *m/z* 468 $[M+45]^-$. After isolation and fragmentation of *m/z* 468 the daughter ion *m/z* 422 $[M-H]^-$ fragmentation pattern exhibits MS/MS product ions at *m/z* 378, 216 and 203. The MS^3-spectrum of a reference probe spiked with **2** (figure 5) corresponds to latter fragmentation pattern. The MS^2 data of the hydrate of **1** is shown in figure 6. The formiate adduct $[M+18+45]^-$ at *m/z* 484 was isolated and the following fragmentation revealed **1** as the product ion at *m/z* 420 $[M-H]^-$. Figure 4 represents a full scan of **1** in the range of 8.31-8.41 min.

Literature:

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Acknowledgement:

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