Identification of the Cytochrome P450 and other enzymes involved in the \textit{in vitro} oxidative metabolism of a novel antidepressant, Lu AA21004

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Running title page

Enzymes involved in the metabolism of Lu AA21004 in vitro

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List of abbreviations: CYP: Cytochrome, HLM: human liver microsomes, Lu AA21004: 1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine, M8: 4-hydroxy-phenyl metabolite of Lu AA21004, M4a: sulfoxide, M0: benzoic acid metabolite of Lu AA21004, M4b: glucuronide of M0.
ABSTRACT

Lu AA21004 (1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is a novel antidepressant and is currently in late stage clinical development for major depressive disorder. In the present study, the metabolism of Lu AA21004 was investigated using human liver microsomes (HLM), human liver S9-fraction and recombinant enzymes. Lu AA21004 was found in vitro to be oxidised to a 4-hydroxy-phenyl metabolite, a sulfoxide, an N-hydroxylated piperazine and a benzylic alcohol, which was further oxidised to the corresponding benzoic acid (Lu AA34443). The formation of the 4-hydroxy-phenyl metabolite was catalysed by CYP2D6 with some contribution from CYP2C9, whereas the formation of the sulfoxide was mediated by CYP3A4/5 and CYP2A6. CYP2C9 and CYP2C19 were the primary enzymes responsible for formation of the N-hydroxylated metabolite. The benzylic alcohol was formed by CYP2D6 only. The oxidation of the benzylic alcohol to the corresponding benzoic acid of Lu AA21004 was catalysed by alcohol dehydrogenase and aldehyde dehydrogenase, with some contribution from aldehyde oxidase. CYP2D6 was also capable of catalyzing the formation of the benzoic acid of Lu AA21004; however, its overall contribution to this pathway was negligible. Enzyme kinetic parameters revealed that the rate-limiting step in the formation of the benzoic acid from Lu AA21004 is the formation of the corresponding alcohol. Thus, the intrinsic clearance ($V_{max}/K_m$) in HLM for metabolism of Lu AA21004 to the benzylic alcohol was $1.13 \times 10^{-6}$ L/min/mg, whereas the subsequent metabolism of the benzylic alcohol to the benzoic acid of Lu AA21004 is characterised by an intrinsic clearance ($V_{max}/K_m$) in S9 fraction of $922 \times 10^{-6}$ L/min/mg.
Introduction

Lu AA21004 (1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is a multi-modal antidepressant that is thought to work through a combination of two pharmacological modes of action: reuptake inhibition and receptor activity. In vitro studies indicate that Lu AA21004 is a 5-HT3\textsuperscript{a} and 5-HT7\textsuperscript{b} receptor antagonist, 5-HT1A\textsuperscript{c} receptor agonist, 5-HT1B\textsuperscript{d} receptor partial agonist and inhibitor of the 5-HT\textsuperscript{e} transporter. In vivo nonclinical studies have demonstrated that Lu AA21004 enhances levels of the neurotransmitters serotonin, noradrenaline, dopamine, acetylcholine and histamine in specific areas of the brain. Data from the nonclinical studies suggest that the multi-modal activity of Lu AA21004 is likely to contribute to its mechanism of action and therapeutic activity (Bang-Andersen et al. 2011, Artigas et al. 2009). Lu AA21004 is currently in clinical development for major depressive disorder. In the nonclinical ADME studies Lu AA21004 is eliminated by extensive metabolic conversion via multiple pathways and metabolites are excreted both via bile and urine (unpublished data). In the human metabolism and excretion study using [\textsuperscript{14}C]-labeled Lu AA21004, six metabolites were quantified in plasma. The major metabolites in human plasma were the benzoic acid of Lu AA21004, M0 (Lu AA34443), and its glucuronide, M4(b). Other metabolites detected in human plasma were a 4-hydroxy-phenyl metabolite, M8, which is further conjugated to its glucuronide, M3, a sulfoxide, M4(a), and two glucuronides, M11 and M12, of the N-hydroxylated Lu AA21004 (unpublished data, and Uldam et al (2011)). The primary metabolites in excreta were the benzoic acid metabolite of Lu AA21004, M0, and its glucuronide conjugate, M4(b), accounting for the vast majority of the dose administered (unpublished data). The aim of the present study was to identify the enzymes involved in the phase 1 metabolism of Lu AA21004 including the intermediates namely the N-
hydroxy piperazine and the benzylic alcohol of Lu AA21004. The enzymes responsible for the formation of the individual metabolites formed following incubation of Lu AA21004 was determined using human recombinant FMO3 and CYP450 enzymes and phenotyped human liver microsomes. Furthermore, a detailed study with determination of enzyme kinetic parameters were performed on the transformation of Lu AA21004 via the benzylic alcohol to the benzoic acid, M0, in order to determine the most important enzymes catalysing this pathway i.e., determining the rate limiting step. The in vitro studies described herein were conducted in accordance with the FDA draft guideline on the conduct of in vitro metabolism studies (FDA, 2006) and the principles described by Tucker et al. (2001), Bjornsson et al. (2003) and Huang et al. (2008).
Materials and Methods

Chemical and reagents

Lu AA21004 HBr (1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine HBr), benzylic alcohol of Lu AA21004, M0 (benzoic acid of Lu AA21004; Lu AA34443), M4(a) (sulfoxide of Lu AA21004), M8 (4-hydroxy-phenyl of Lu AA21004 sodium salt) and the N-hydroxylated intermediate of Lu AA21004 were prepared at H. Lundbeck A/S as described in supplemental data (Supplemental Synthesis of Compounds) and by Uldam et al (2011). [14C]-Lu AA21004 HBr was purchased from BioDynamics (now Quotient Bioresearch), United Kingdom, NADPH, NAD+, NADP+, isocitric acid, isocitric dehydrogenase, KCl, MgCl2, Tris-HCl, KH2PO4, raloxifene, allopurinol, and disulfiram were purchased from Sigma, St. Louis, MO and 4-methylpyrazole was purchased from ACROS, Belgium. Microsomes from human B-lymphoblastoid AHH-1 cell line expressing human CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4, control microsomes from human B-lymphoblastoid AHH-1 cell line containing only the vector, microsomes from baculovirus infected insect cells (BTI-TN-5B1-4) expressing human FMO3, and control microsomes from insect cells (BTI-TN-5B1-4) infected with wild type baculovirus (Autographa californica), and pooled human liver S9 (pool of 50 donors) were all purchased from Gentest Corporation, Woburn, MA. HepatoSomes control pool, Lot 2.0 (pooled human liver microsomes), Hepatoscreen Test Kit with 15 donors (phenotyped human liver microsomes) was purchased from Human Biologics International (HBI), Scottsdale, AZ. Each donor was enzymatically characterised by the supplier with respect to CYP isoform selective reactions as follows (isoenzyme in parenthesis): caffeine N3-demethylation (CYP1A2), coumarin 7-hydroxylation.
(CYP2A6), S-mephenytoin N-demethylation (CYP2B6), tolbutamide methyl hydroxylation (CYP2C9), S-mephenytoin-4'-hydroxylation (CYP2C19),
dextromethorphan O-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1),
dextromethorphan N-demethylation (CYP3A), testosterone 6β-hydroxylation (CYP3A),
lauric acid 12-hydroxylation (CYP4A11). Pooled human liver microsomes (pool of 50 donors) and a Reaction Phenotyping Kit with 16 donors (phenotyped human liver microsomes) were purchased from XenoTech, Lenexa, KS. Each donor was enzymatically characterised by the supplier with respect to CYP isoform selective reactions as follows (isoenzyme in parenthesis): 7-ethoxyresorufin O-dealkylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), S-mephenytoin N-demethylation (CYP2B6), paclitaxel (Taxol®) 6α-hydroxylation (CYP2C8), diclofenac 4'-hydroxylation (CYP2C9), S-mephenytoin-4'-hydroxylation (CYP2C19),
dextromethorphan O-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1),
testosterone 6β-hydroxylation (CYP3A), lauric acid 12-hydroxylation (CYP4A11), benzydamine N-oxidation (FMO3).

Metabolite identification following incubation with Lu AA21004

In order to investigate which metabolites were formed following incubation of Lu AA21004 in pooled human liver microsomes, 0.5 and 1.0 mg protein/ml microsomes were incubated at 37 °C in 50 mM Tris-HCl pH =7.4, 154 mM KCl, 5 mM MgCl₂, and an NADPH-regenerating system (1 mM NADP⁺, 5 mM isocitric acid, and 1 U/ml isocitric dehydrogenase) in a final volume of 0.5 ml. Following a 5 min pre-incubation, the reaction was initiated by adding the substrate (100 μM final concentration), which was dissolved in water:acetonitrile; 1:1 resulting in a final concentration of acetonitrile of 0.5% in the incubation. The incubation times were 0,
30, and 180 min. The reaction was terminated by the addition of 1 ml ice cold acetonitrile containing internal standard. The samples were then centrifuged at ca. 4000 x g and the supernatant was evaporated to dryness under N₂ at 25 °C. The residue was dissolved in 125 μl 10mM ammonium acetate pH = 5: acetonitrile; 9:1, and the ca. 18000 x g supernatant was analysed by HPLC-MS-MS. The samples were analysed using Method A (please refer to Analysis section below).

**NADPH-dependency following incubation with Lu AA21004**

The NADPH-dependency of the metabolism of Lu AA21004 was examined by incubating 0.5 mg protein/ml pooled human liver microsomes for 0 and 180 min with A) NADPH-regenerating system, B) NADPH regenerating system without NADP⁺, and C) without NADPH regenerating system. The incubation conditions and sample preparation was the same as described in Metabolite Identification. The samples were analysed using Method A (please refer to Analysis section below).

**Enzyme kinetics following incubation of Lu AA21004**

The incubation conditions were the same as described in Metabolite Identification. The protein concentration was 0.5 mg/ml and the final volume was 0.5 ml. Following a 10 min. pre-incubation, the reaction was initiated by adding [¹⁴C]-Lu AA21004 (1 - 500 μM), which was dissolved in ethanol, resulting in a final concentration of ethanol of 1 %. The level of radioactivity was dependent on the specific activity and was in the range 0.07 μCi/ml to 0.4 μCi/ml. The incubation time was 30 min. The reaction was terminated by the addition of 0.5 ml ice cold acetonitrile containing internal standard. The samples were then centrifuged at ca. 4000 x g and the supernatant was evaporated to dryness under N₂ at 20° C overnight. The residue was dissolved in 250 μl reconstitution buffer (methanol: ammonium formate 50 mM (pH 3.5); 6:4) and
analysed by HPLC-MS/MS and on-line radioactivity detector using Method B (please refer to Analysis section below). The enzyme kinetic investigations were performed within the range of linearity with respect to protein concentration and incubation time, which were determined in a preliminary experiment.

Incubation of Lu AA21004 with recombinant enzymes

Recombinant human liver FMO3 and CYP isoenzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) were incubated with 8 μM and 50 μM Lu AA21004. The experimental procedure was the same as for the Metabolite Identification experiment. The protein content was 1 mg/ml as suggested by the supplier, and the incubation time was 120 min. As control, microsomes from the same cell line containing only the vector were used. The samples were analysed by Method A (please refer to Analysis section below).

Incubation of Phenotyped human liver microsomes with Lu AA21004

Microsomes from each of the 15 phenotyped donors were incubated under the same conditions as for the Metabolite Identification experiment. The protein concentration was 0.5 mg/ml and the incubation time was 0 and 60 min. The concentrations of Lu AA21004 were 8 μM and 50 μM. The samples were analysed by Method A (please refer to Analysis section below). The incubation conditions were performed within the range of linearity with respect to protein concentration and incubation time for pooled human liver microsomes, which were determined in a preliminary experiment. The results obtained were subjected to correlation analysis.
NADPH-dependency of the metabolism of the benzylic alcohol of Lu AA21004 to the benzoic acid of Lu AA21004 in human liver microsomes

The NADPH-dependency of the metabolism of the benzylic alcohol of Lu AA21004 to the corresponding benzoic acid was examined by incubating 50 μM of the benzylic alcohol with 0.5 mg protein/ml pooled human liver microsomes for 0 min and 180 min with A) NADPH-regenerating system, B) NADPH regenerating system without NADP⁺, C) without NADPH regenerating system and D) Tris-buffer only (no microsomes). The incubation conditions and sample preparation was according to the method described for the kinetics experiment with the benzylic alcohol in human liver microsomes. The samples were analysed using HPLC Method B (please refer to Analysis section below).

Enzyme kinetics in human liver microsomes following incubation with the benzylic alcohol of Lu AA21004

Pooled human liver microsomes were incubated at 37 °C in Tris-buffer containing 50 mM Tris-HCl, 154 mM KCl, 5 mM MgCl₂, and an NADPH-regenerating system (1 mM NADP⁺, 5 mM isocitric acid, and 1 U/ml isocitric dehydrogenase). The protein concentration was 0.5 mg/ml and the final volume was 0.5 ml. Following a 10 min. pre-incubation, the reaction was initiated by adding the benzylic alcohol of Lu AA21004 (1 - 500 μM), which was dissolved in water:acetonitrile resulting in a final concentration of acetonitrile of 0.5 % in the incubation. The incubation time was 20 min. The reaction was terminated by the addition of 0.5 ml ice cold acetonitrile containing internal standard. The samples were then centrifuged at ca. 4000 x g and the supernatant was evaporated to dryness under N₂ at 20° C over night. The residue...
was dissolved in 250 μl reconstitution buffer (methanol: ammonium formate 50 mM (pH 3.5); 6:4) and analysed by HPLC-MS/MS using HPLC Method B (please refer to Analysis section below). The enzyme kinetic investigations were performed within the range of linearity with respect to protein concentration and incubation time, which were determined in a preliminary experiment.

**Incubation of recombinant enzymes with the benzylic alcohol of Lu AA21004**

Recombinant human liver FMO3 and CYP isoenzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) were incubated with 8 and 50 μM benzylic alcohol of Lu AA21004. The experimental procedure was the same as for the kinetics experiment of the benzylic alcohol of Lu AA21004 with human liver microsomes. The incubation time was 120 min. As a control, microsomes from the same cell line containing only the vector were used. The samples were analysed by HPLC-MS/MS using Method B (please refer to Analysis section below).

**Incubation of phenotyped human liver microsomes with the benzylic alcohol of Lu AA21004**

Human liver microsomes from each of the phenotyped 15 donors were incubated under the same conditions as for the kinetic experiment with the benzylic alcohol of Lu AA21004 in human liver microsomes. The incubation time was 15 min. The concentrations of the benzylic alcohol of Lu AA21004 were 8 and 50 μM. The samples were analysed by Method B (please refer to Analysis section below). The incubation conditions were performed within the range of linearity with respect to protein concentration and incubation time for pooled human liver microsomes, which
were determined in a preliminary experiment. The results obtained were subjected to correlation analysis.

**Cofactor-dependency of the metabolism of the benzylic alcohol to the benzoic acid of Lu AA21004 in human liver S9 fraction**

The NAD⁺ and NADPH-dependency on the metabolism of the benzylic alcohol of Lu AA21004 to the corresponding acid in human liver S9 fraction was examined by incubating 8 and 50 μM benzylic alcohol of Lu AA21004 with 0.02 mg protein/ml for 15 min. In addition to the substrate, the incubations contained: A) S9 fraction and NADPH, B) S9 fraction and NAD⁺, C) S9 fraction only, D) potassium phosphate buffer. The incubation conditions and sample preparation was according to the method described for the kinetics experiment in human liver S9 fraction. The samples were analysed by HPLC-MS/MS using HPLC Method B (please refer to Analysis section below).

**Enzyme kinetics of the metabolism of the benzylic alcohol to the benzoic acid of Lu AA21004 in human liver S9-fraction**

Pooled human liver S9-fraction was incubated at 37 °C in potassium phosphate buffer containing 50 mM KH₂PO₄, pH = 7.4 and 1 mM NAD⁺. The protein concentration was 0.02 mg/ml and the final volume was 0.5 ml. Following a 10 min pre-incubation, the reaction was initiated by adding the benzylic alcohol of Lu AA21004 (1 μM - 500 μM), which was dissolved in water:acetonitrile resulting in a final concentration of acetonitrile of 0.5 % in the incubation. The incubation time was 20 min. The reaction was terminated by the addition of 0.5 ml ice cold acetonitrile containing internal standard. The samples were then centrifuged at ca. 4000 x g and the supernatant was evaporated to dryness under N₂ at 25° C. The residue was dissolved in 250 μl.
reconstitution buffer (methanol: ammonium formate 50 mM (pH 3.5); 6:4) and analysed by HPLC-MS/MS using Method B (please refer to Analysis section below). The enzyme kinetic investigations were performed within the range of linearity with respect to protein concentration and incubation time.

**Incubation of pooled human liver S9 fraction with the benzylic alcohol of Lu AA21004 and chemical inhibitors**

Pooled human liver S9 fraction was incubated with one of the following enzyme inhibitors (concentration and enzyme in parenthesis): raloxifene (100 nM, aldehyde oxidase), Allopurinol (100 µM, xanthine oxidase), disulfiram (40, 100 and 500 µM, aldehyde dehydrogenase) and 4-methylpyrazole (600 µM, alcohol dehydrogenase) (Obach 2004a, Obach 2004b, Dawidek-Pietryka 1998, Walsh 2002, Kassam 1989, Lam 1997, Klyosov 1996 and Okamoto 2003). The protein content was 0.02 mg /ml and incubation time was 15 min. The inhibitors were dissolved in DMSO resulting in a final DMSO concentration of 1 % in the incubations. Controls contained only the solvent (1 % DMSO) and were pre-incubated for 10 min except for incubations containing for allopurinol and disulfiram where the pre-incubation time was 30 min to allow time-dependent inhibition. The concentration of substrate was 2 μM (equal to Kₘ), 8 μM and 50 μM. The procedure was the same as for the kinetics experiment in human liver S9-fraction. The samples were analysed by HPLC-MS/MS using Method B (please refer to Analysis section below).

**Analysis**

**Method A:** Chromatographic separation was obtained using a Waters 2790 HPLC system and Waters Symmetry C8 guard column and column (3.9 x 20 mm and 3.9 x 50mm, respectively). Mobile phase A consisted of 10 mM ammonium acetate buffer,
pH 5.0, whereas mobile phase B consisted of 10 ml 10 mM ammonium acetate buffer, pH 5.0 in 1L acetonitrile. The temperature of the column oven was 40 °C and the flow rate was 1 ml/min or 250 µl/min. For the first two minutes the eluent consisted of 10 % mobile phase B followed by a linear gradient to 80 % mobile phase B in 13 minutes. Mobile phase B was then maintained at 80 % for 5 minutes before re-equilibration for 5 minutes. Total run time was 27 minutes. The eluent was coupled to a Micromass Q-TOF 2 mass spectrometer or a Finnigan TSQuantum mass spectrometer. Positive electrospray ionisation (ES+) methods were used (TOF Scan, TOF-MS-MS and single reaction monitoring (SRM)). The SRM channels were as follows: 299>150 (Lu AA21004), 315>192 (benzylic alcohol of Lu AA21004), 315>191 (M4a), 315>166 (M8), 315>256 (N-hydroxylated metabolite), 329>286 (M0), 325>109 (internal standard). The MS response was linear at least in the range 10 nM - 2.5 μM for Lu AA21004, M0, M4a, and the benzylic alcohol of Lu AA21004. Due to lack of standard for M8 and the N-hydroxylated piperazine, the assumption was that the MS responses for the two metabolites were linear in the same range. Repeatability was tested before each set of analysis by injecting a suitability test (Lu AA21004 and available metabolite standards (see above)). The suitability test was be injected several times before the samples were analysed. The accept criteria following three succeeding injections were: Retention time: CV \leq 5 \% for each standard. Area: CV \leq 20 \% for each standard. The suitability test was also injected several times during the analysis of the samples the acceptance criteria of the run being as above.

**Method B:** Chromatographic separation was obtained using a Surveyor HPLC system and Waters Xterra guard column (4.6 x 10mm) and Waters Luna 5u phenyl - hexyl column (4.6 x 250 mm). Mobile phase A consisted of 50 mM ammonium formate pH
3.5: acetonitrile (9:1 v/v) whereas mobile phase B consisted of 50 mM ammonium formate pH 3.5: acetonitrile (1:9 v/v). The temperature of the column oven was 40 °C and the flow rate was 1 ml/min. A linear gradient (1 ml/min) was applied (0 to 11.0 minutes) from 88% to 70% mobile phase A and was followed by 70 % mobile A from 11.0 to 15.0 minutes. Then a linear gradient (15.0-15.1 minutes) from 70% to 60% mobile phase A was applied followed by another linear gradient (15.1-20 minutes) from 60% to 50% mobile phase A. The column was then washed for 5 min. with 5 % mobile phase A and re-equilibrated for 5.4 min. (total run time 31 minutes). The eluent was coupled to a Flo One radiodetector and a Finnigan TSQuantum mass spectrometer. Positive electrospray ionisation (ES+) method was used. The single reaction monitoring channels were as in Method A except for the benzylic alcohol of Lu AA21004, where the SRM was 315>150. This method was further modified to quantify the benzoic acid of Lu AA21004 following incubation with the benzylic alcohol of Lu AA21004 resulting in total run time of 14 minutes. Samples were analysed quantitatively for the benzoic acid of Lu AA21004 by HPLC-MS/MS. The calibration curve was prepared by spiking microsomes or S9 fractions with increasing concentrations of the benzoic acid of Lu AA21004 followed by the same sample preparation as for the authentic samples. The MS response was linear at least in the range 5 nM - 20 μM for the benzoic acid of Lu AA21004. Repeatability was tested before each set of analysis by injecting a suitability test (Lu AA21004 and available metabolite standards (see above)). The suitability test was be injected several times before the samples were analysed. The accept criteria following three succeeding injections were: Retention time: CV ≤ 5 % for each standard. Area: CV ≤ 20 % for each standard. The suitability test was also injected several times during the analysis of the samples the acceptance criteria of the run being as above.
Data analysis

Due to sensitivity in the radiodetector, only the benzylic alcohol of Lu AA21004 and M4(a) could be quantified. Therefore the area of the metabolite/area of internal standard (ISD) was used (as described in Method A and Method B). The areas of peaks observed in 0 min incubations (HLM) or in controls (recombinant enzymes) were subtracted from the test samples. For the enzyme kinetics, [14C]-labelled Lu AA21004 was used and the benzylic alcohol and the sulfoxide of Lu AA21004 analysed quantitatively using radioactivity.

The amount of benzoic acid formed following incubation with the benzylic alcohol was determined using a calibration curve as described in Method B.

The apparent kinetic parameters, $K_m$ and $V_{max}$, were calculated by non-linear regression using GraphPad Prism Vers. 4.01. The data were fitted to a one enzyme Michaelis-Menten equation ($V = V_{max} x [S]/(K_m + [S])$) or a Michaelis-Menten model with uncompetitive substrate inhibition ($V = V_{max} x [S]/(K_m + [S] + ([S]^2/K_s)$) [Venkatakrishnan et al 2001]. Eadie-hofstee transformations were made to investigate any indications of involvement of multiple enzymes.

Data obtained from phenotyped human liver microsomes were subjected to linear regression analysis using Microsoft Excel 2000. The data used was the average of triplicate determinations. An F-test was used to test whether the slope was significantly different from 0. Data from the experiment using inhibitors were analysed by F-tests to assure homogeneity of variance followed by unpaired t-tests. When variances were different an unpaired t-test with Welch correction was used. All analysis was performed using GraphPad Prism Vers. 4.01.
Results

**Metabolite identification following incubation with Lu AA21004**

Incubation of pooled human liver microsomes with Lu AA21004 resulted in the formation of benzylic alcohol, benzoic acid (M0), sulfoxide (M4a), 4-hydroxy-phenyl metabolite (M8) and an N-hydroxylated piperazine of Lu AA21004. Furthermore, three metabolites with m/z = 331 were also formed indicative of dihydroxylated metabolites of Lu AA21004 or monohydroxylated sulfoxides. Metabolites with m/z = 331 were not detected the *in vivo* human metabolite radio-profiling study (unpublished data) and are therefore not further discussed. An overview of the *in vitro* metabolic pathway is shown in Figure 1. No metabolism was observed in the absence of NADPH-regenerating system or in the presence of the regenerating system without NADP⁺ (data not shown). Thus, all of the metabolites formed were dependent on the presence of NADPH.

**Incubation of Lu AA21004 with recombinant enzymes**

Lu AA21004, following incubation with recombinant enzymes, was oxidised to the benzylic alcohol of Lu AA21004, its corresponding benzoic acid, a sulfoxide, an N-hydroxylated piperazine and a 4-hydroxy-phenyl metabolite. CYP2D6 showed the highest catalytic activity with respect to the formation of the benzylic alcohol of Lu AA21004 (normalised with respect to the amount of CYP450). CYP2C9 and CYP2C19 also had the capability to form the benzylic alcohol of Lu AA21004, although with a lower catalytic activity (Figure 2). CYP2D6 was the only CYP isozyme catalysing the formation of the benzoic acid of Lu AA21004 (Figure 2). With respect to the sulfoxide of Lu AA21004, CYP3A4 as well as CYP2A6 and CYP2C8
were capable of catalysing the formation of this metabolite, with CYP3A4 having the highest catalytic activity (Figure 2). The formation of the N-hydroxylated piperazine was catalysed by CYP2C9, CYP2C19 and to a much lower extent by CYP2B6 (Figure 2). Recombinantly expressed CYP2D6 showed the highest catalytic activity, whereas CYP2C9 and CYP2C19 showed some catalytic activity with respect to the formation of the 4-hydroxy-phenyl metabolite (Figure 2). None of the other recombinant enzymes were able to metabolise Lu AA21004 to the 4-hydroxy-phenyl metabolite under the conditions used. FMO3 showed no or very little catalytic activity with respect to the formation of any of the metabolites (data not shown).

**Incubation of phenotyped human liver microsomes with Lu AA21004**

Lu AA21004, following incubation with phenotyped human liver microsomes, was oxidised to a benzylic alcohol of Lu AA21004, its corresponding benzoic acid, a sulfoxide, an N-hydroxylated piperazine and 4-hydroxy-phenyl metabolite. The formation of the benzylic alcohol correlated with CYP2D6 and CYP2C9 activities (Table 1). With respect to the formation of the benzoic acid of Lu AA21004, correlation was only observed with CYP2D6 activity. The metabolism of Lu AA21004 to the sulfoxide metabolite correlated with CYP2A6, CYP2B6, and CYP3A activities. Furthermore, at a substrate concentration of 50 μM the formation correlated with CYP2E1 activity. At a substrate concentration of 8 μM, the formation of the N-hydroxylated piperazine correlated with CYP2A6, CYP2B6, CYP2C9, CYP2C19 and CYP3A activities. However, using a substrate concentration of 50 μM resulted in correlation only with CYP2A6, CYP2B6 and CYP3A activities. The metabolism of Lu AA21004 to 4-hydroxy-phenyl metabolite correlated with CYP2D6 enzyme activity. Some correlation was obtained with CYP2C9 enzyme activity but only at a
substrate concentration of 8 μM (Table 1). Inter-correlation existed between the following enzyme activities: CYP2A6 and CYP2B6, CYP2A6 and CYP3A, CYP2B6 and CYP3A, CYP2C9 and CYP3A, CYP2C19 and CYP3A (Table 1).

**Formation of the benzoic acid of Lu AA21004 from the benzylic alcohol of Lu AA21004**

The second step in the biotransformation of Lu AA21004 to the benzoic acid metabolite was investigated using the benzylic alcohol of Lu AA21004 as a substrate. The benzoic acid of Lu AA21004 was formed following incubation of the benzylic alcohol with human liver microsomes and human liver S9 fraction (data not shown). NADPH was not required for the metabolism of the benzylic alcohol to its corresponding benzoic acid in human liver microsomes. Thus, the metabolism of the benzylic alcohol to the benzoic acid of Lu AA21004 in human liver microsomes occurred in the absence of NADPH. However, the amount of benzoic acid of Lu AA21004 formed increased with a factor of two with the presence of NADPH, indicating that the formation of this metabolite is catalysed by NADPH-dependent as well as NADPH-independent enzymes (data not shown). In human liver S9-fraction, the benzylic alcohol of Lu AA21004 was metabolised to its corresponding benzoic acid metabolite to some extent in the absence of cofactors. The amount of benzoic acid of Lu AA21004 formed was not affected by the presence of NADPH, indicating that the contribution of CYP450 under those conditions is negligible. The presence of NAD⁺ increased the formation of the benzoic acid by a factor of 9 and 7 at substrate concentration of 8 μM and 50 μM respectively (data not shown), indicating that alcohol dehydrogenase and/or aldehyde dehydrogenase are probably the major enzymes responsible for the formation of the benzoic acid.
Incubation of recombinant enzymes with the benzylic alcohol of Lu AA21004

The benzylic alcohol of Lu AA21004 was oxidised to its corresponding benzoic acid following incubation with recombinant enzymes. CYP2D6 was the only enzyme catalysing the formation of the benzoic acid metabolite (data not shown).

Incubation of phenotyped human liver microsomes with benzylic alcohol of Lu AA21004

The benzylic alcohol of Lu AA21004 was also oxidised to its corresponding acid following incubation with phenotyped human liver microsomes. However, no correlation between the formation of the benzoic acid metabolite and any of the CYP isozyme activities was obtained (data not shown). Thus, enzymes other than CYP450 seem to participate in this biotransformation, as a high correlation with CYP2D6 activity would be expected if only CYP450 was involved (based on the results from the recombinant enzymes, in which only CYP2D6 showed catalytic activity). It should, however, be kept in mind that the microsomes are contaminated with other organelles.

Incubation of pooled human liver S9-fraction with benzylic alcohol of Lu AA21004 and chemical inhibitors

Pooled human liver S9-fraction was incubated with four different inhibitors at three substrate concentrations. In addition to the 8 µM and 50 µM used for the other assays, 2 µM of the benzylic alcohol of Lu AA21004 was also included in this inhibitor assay to include a substrate concentration equal to $K_m$. Allopurinol did not inhibit the metabolism of the benzylic alcohol of Lu AA21004 to its corresponding acid at any of the substrate concentrations used (Figure 3). At the substrate concentration equal to
K_m, the metabolism was inhibited approximately 40 % by disulfiram and raloxifene, whereas 4-methylpyrazole inhibited the reaction by approximately 60 %. At substrate concentrations of 8 µM and 50 µM, disulfiram inhibited the metabolism more than 80 %. Raloxifene inhibited the metabolism by ca. 45 % and 35 % at substrate concentrations of 8 µM and 50 µM, respectively, whereas 4-methylpyrazole inhibited ca. 55 % and 35 % at substrate concentrations of 8 µM and 50 µM, respectively. This indicates that at low substrate concentration, alcohol dehydrogenase, aldehyde dehydrogenase and aldehyde oxidase are involved to the same extent in the metabolism of the benzylic alcohol of Lu AA21004 to the benzoic acid metabolite, whereas at higher substrate concentrations, aldehyde dehydrogenase is the major enzyme responsible for this metabolism, with some contribution from alcohol dehydrogenase and aldehyde oxidase. Xanthine oxidase is not involved in the metabolism of the benzylic alcohol of Lu AA21004 to its corresponding benzoic acid under the conditions used in this study, since allopurinol did not inhibit this biotransformation.

**Enzyme kinetics following incubation of Lu AA21004**

The enzyme kinetics following incubation of Lu AA21004 was investigated in human liver microsomes. The benzylic alcohol and the sulfoxide of Lu AA21004 were detected by on-line radioactivity detection over the entire concentration range of Lu AA21004 (1 – 500 µM), whereas the sensitivity for the two other metabolites, 4-hydroxy-phenyl and the N-hydroxylated piperazine, was too low. Therefore, the K_m for the two latter metabolites was estimated using MS/MS. The best fit of data was obtained using the simple Michaelis-Menten equation. Under the incubation conditions used in this study, the K_m and V_max for the formation of the benzylic alcohol of Lu AA21004 in pooled human liver microsomes was estimated to 231 µM.
and 262 pmol benzylic alcohol of Lu AA21004/min/mg protein (Figure 4). Eadie Hofstee transformation did not reveal any evidence of two-enzyme kinetics. $V_{\text{max}}$ with respect to the formation of the sulfoxide of Lu AA21004 was not reached under the conditions used in this study with substrate concentrations up to 500 µM. The data set was therefore subjected to non-linear regression, setting $V_{\text{max}}$ to $\geq$ velocity obtained at 500 µM resulting in $K_{\text{m}} \geq 208$ µM (Figure 4). The Michaelis-Menten constant $K_{\text{m}}$ for the formation of 4-hydroxy-phenyl metabolite and the N-hydroxylated piperazine of Lu AA21004 were 323 µM and 300 µM, respectively (Figure 4).

**Enzyme kinetics following incubation with benzylic alcohol of Lu AA21004**

The enzyme kinetics of the formation of the benzoic acid of Lu AA21004 from its corresponding alcohol, the benzylic alcohol, was investigated in human liver microsomes and human liver S9-fraction in order to determine the rate limiting step in the biotransformation of Lu AA21004 to the benzoic acid metabolite, the major metabolic pathway in humans. Data obtained following incubation of pooled human liver microsomes with increasing concentrations of the benzylic alcohol of Lu AA21004 were fitted to a Michaelis-Menten model with uncompetitive substrate inhibition using non-linear regression to obtain the best fit. The resulting $K_{\text{m}}$, $K_{s}$ and $V_{\text{max}}$ estimates were 95 µM, 275 µM and 59 pmol/min/mg protein, respectively, for the formation of the benzoic acid of Lu AA21004 (Figure 5). Data obtained following incubation of pooled human liver S9-fraction with increasing concentrations of the benzylic alcohol were fitted to the Michaelis-Menten equation using non-linear regression resulting in $K_{\text{m}}$ and $V_{\text{max}}$ estimates of 1.7 µM and 1567 pmol/min/mg protein, respectively, for the formation of the benzoic acid metabolite (Figure 5).
Eadie-Hofstee transformation did not reveal any evidence of more than one enzyme involved in the biotransformation, indicating that the enzymes involved in this biotransformation have similar Km values towards the benzylic alcohol of Lu AA21004. An overview of enzyme kinetic parameters is shown in Table 2.
Discussion

The enzymes involved in the *in vitro* metabolism of Lu AA21004 were investigated. Four phase one metabolites are of clinical relevance due to the observation of the metabolites or their glucuronide conjugates in human plasma following oral administration of Lu AA21004. The four metabolites are the sulfoxide, the N-hydroxylated piperazine, the 4-hydroxy phenyl-metabolite, and the benzoic acid. In addition, the benzylic alcohol was shown in this study to be precursor for the benzoic acid metabolite and was therefore included. An overview of the results obtained is shown in Figure 1.

Using recombinant enzymes, only CYP3A4 and CYP2A6 were capable of catalysing the formation of the sulfoxide. In the correlation analysis, a correlation was obtained with CYP3A, CYP2A6 and CYP2B6 and at high substrate concentration also some correlation was obtained with CYP2E1. As inter-correlation existed between CYP2A6 and CYP2B6, and CYP2B6 and CYP3A and since recombinant CYP2B6 and CYP2E1 did not show any catalytic activity with respect to the formation of the sulfoxide, CYP3A and CYP2A6 are expected to be the primary enzymes involved. The formation of the N-hydroxylated piperazine seems to be catalysed primarily by CYP2C9 and CYP2C19. In addition, recombinantly expressed CYP2B6 showed little catalytic activity and tested also positive in the correlation analysis. The latter may be due to inter-correlation with other isoenzymes. However, the involvement of CYP2B6 cannot be excluded. With respect to the formation of the 4-hydroxy-phenyl metabolite, recombinant CYP2D6 showed the highest catalytic activity, whereas CYP2C9 and CYP2C19 showed some catalytic activities. Furthermore, the metabolism of Lu AA21004 to the 4-hydroxy-phenyl metabolite correlated with CYP2D6 enzyme activity. Some correlation was also obtained with CYP2C9 enzyme
activity but only at a substrate concentration of 8 μM. No correlation was obtained with other enzyme activities. Thus the metabolism of Lu AA21004 to the 4-hydroxy-phenyl metabolite is catalysed by CYP2D6, but CYP2C9 may also be involved to a minor extent. The major enzyme responsible for the formation of the benzylic alcohol (precursor for the benzoic acid metabolite) is CYP2D6 whereas CYP2C9 seems to contribute to a minor extent.

The benzoic acid metabolite was also detected following incubation of Lu AA21004 with human liver microsomes as well as recombinant enzymes. Under those incubation conditions, CYP2D6 seems to be the only enzyme catalysing the formation of this metabolite. In order to investigate the capability of CYP450 and FMO3 to catalyse the metabolism of the benzylic alcohol to the corresponding benzoic acid, the benzylic alcohol was incubated with recombinant enzymes as well as CYP450 and FMO3 phenotyped human liver microsomes. Recombinant CYP2D6 was the only enzyme catalysing the formation of the benzoic acid metabolite and as expected, recombinant FMO3 did not show any catalytical capability towards this biotransformation. With respect to the phenotyped human liver microsomes, no correlation was obtained. This is probably due to the contribution from NADPH independent enzymes (and thus non-CYP). To investigate whether other enzymes than CYP450 were involved in the metabolism of the benzylic alcohol to its corresponding benzoic acid, the benzylic alcohol was incubated with human liver S9-fraction and the dependency of the cofactors NADPH and NAD⁺ was investigated. Some metabolism occurred without any cofactor present, which could indicate the involvement of enzymes such as aldehyde oxidase and xanthine oxidase. The amount of the benzoic acid formed was independent on the presence of NADPH, indicating that contribution of CYP450 to the metabolism of the benzylic alcohol to corresponding acid is
negligible in the S9-fraction. When adding NAD\(^+\) to the incubation mixture, the metabolism of the benzylic alcohol to the benzoic acid metabolite was increased by a factor of 7 to 9. Thus NAD\(^+\) dependent enzymes were the major enzymes responsible for the metabolism of the benzylic alcohol to its corresponding acid *in vitro*. Alcohol dehydrogenase and aldehyde dehydrogenase are NAD\(^+\)-dependent enzymes.

The metabolism of the benzylic alcohol to the benzoic acid was inhibited following incubation with 4-methylpyrazole and disulfiram, inhibitors of alcohol dehydrogenase and aldehyde dehydrogenase respectively. Furthermore, the aldehyde oxidase inhibitor, raloxifene, also inhibited the metabolism, whereas the xanthine oxidase inhibitor, allopurinol, showed no inhibitory potency towards the metabolism of the benzylic alcohol to the benzoic acid. Based on those results together with the cofactor requirements, alcohol dehydrogenase and aldehyde dehydrogenase are the major enzymes responsible for the metabolism of the benzylic alcohol to its corresponding acid, with some contribution from aldehyde oxidase. Furthermore, CYP2D6 is also capable of catalyzing this reaction; however, the contribution is expected to be less important based on the results mentioned above. This is also supported by the intrinsic clearances \((V_{\text{max}}/K_{m})\), which in HLM was \(0.621 \times 10^{-6}\) L/min/mg protein whereas in human liver S9-fraction the \(V_{\text{max}}/K_{m}\) was \(922 \times 10^{-6}\) L/min/mg protein. The subcellular fractions are not directly comparable; however, the enzymes in HLM are more enriched compared to the S9-fraction. This adds to the argument that the contribution of CYP2D6 can be considered negligible.

Moreover, the metabolism of the benzylic alcohol to its corresponding acid seems to be relatively fast compared to the metabolism of Lu AA21004 to the benzylic alcohol using the same arguments as above. Thus, the intrinsic clearance \((V_{\text{max}}/K_{m})\) in HLM for metabolism of Lu AA21004 to the benzylic alcohol was \(1.13 \times 10^{-6}\) L/min/mg.
whereas the subsequent metabolism of the benzylic alcohol to the benzoic acid is characterised by an intrinsic clearance ($V_{\text{max}}/K_m$) in the S9-fraction of $922 \times 10^{-6}$ L/min/mg. Thus the latter clearance is at least 800 times higher and bearing in mind that the human liver S9-fraction is more diluted than the human liver microsomes, the oxidation of Lu AA21004 to the benzylic alcohol is believed to be the rate limiting step in the formation of the benzoic acid. In conclusion, at least five cytochrome P450 isoenzymes are involved in the metabolism of Lu AA21004 in vitro. Furthermore, the benzylic alcohol of Lu AA21004 is the precursor of the benzoic acid metabolite, a step that was catalysed by alcohol dehydrogenase and aldehyde dehydrogenase, with some contribution from aldehyde oxidase. In addition, CYP2D6 was also capable of catalyzing the formation of the benzoic acid metabolite, however, the contribution is expected to be of less importance.
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Authorship contributions

Participated in research design: Hvenegaard and Dalgaard

Conducted experiments: Hvenegaard, Bang-Andersen, Pedersen, Jørgensen, Püschl

Contributed to new reagents or analytical tools: Bang-Andersen, Jørgensen, Püschl

Performed data analysis: Hvenegaard, Pedersen, Jørgensen, Püschl

Wrote or contributed to the writing of the manuscript: Hvenegaard, Bang-Andersen, Pedersen, Jørgensen, Püschl
References


Footnotes

a: Only tested on genes HTR3A and HTR3B

b: Gene HTR7

c: Gene HTR1A

d: Gene HTR1B

e: Gene SLC6A4
Legends for Figures

Figure 1. Enzymes involved in the in vitro metabolism of Lu AA21004

The isoenzyme written in bold is expected to be the primary enzymes involved.

Enzymes in parentheses may or may not be involved. ADH = alcohol dehydrogenase, ALDH = aldehyde dehydrogenase, AO = aldehyde oxidase.

Figure 2. Formation of metabolites by recombinant human cytochrome P450 isoenzymes following incubation with Lu AA21004

Recombinant human cytochrome P450 isoenzymes were incubated for 120 min. with 8 µM Lu AA21004 and 50 µM Lu AA21004. Data shown is the area obtained in the MS for the given metabolite relative to the internal standard and normalised to the amount of P450 (average of triplicate determinations ± standard deviation) following incubation with 8 µM. Similar results were obtained following incubation with 50 µM.

Figure 3. Incubation of the benzylic alcohol with chemical inhibitors.

Pooled human liver S9 fraction was incubated with chemical inhibitors and 2 µM (Panel A), 8 µM (Panel B) or 50 µM (Panel C) of the benzylic alcohol of Lu AA21004 for 15 minutes and the amount formed of the benzoic acid of Lu AA21004 determined. Vehicle controls contained the solvent used for inhibitors and were pre-incubated for the same time as the inhibitors. Data shown is the mean of triplicate determinations. * p<0.05. ** p<0.01 *** p<0.001.
Figure 4. Enzyme kinetics following incubation with Lu AA21004.

Pooled human liver microsomes (0.5 mg/ml) were incubated with 1 - 500 µM [14C]-Lu AA21004. The benzylic alcohol and the sulfoxide metabolites a was detected by on-line radioactivity detection over the entire concentration range of Lu AA21004 (1 – 500 µM), whereas the sensitivity for the two other metabolites, 4-hydroxy-phenyl Lu AA21004 and N-hydroxy Lu AA21004, was too low using the radiodetector. Therefore, the K_m for the two later metabolites was estimated using areas obtained by MS/MS. The results obtained at 500 µM were omitted for the benzylic alcohol and the sulfoxide metabolites as the duplicate values were very dissimilar. Data shown is the mean of duplicate determinations.

Figure 5. Enzyme kinetics in human liver microsomes or S9 fraction following incubation with the benzylic alcohol.

Pooled human liver microsomes (0.5 mg/ml) (Panel A) or pooled human liver S9 fraction (0.02 mg/ml) (Panel B) were incubated with 1 - 500 µM benzylic alcohol and enzyme kinetic parameters estimated.
Tables

Table 1. Correlation analysis between form selective enzyme activities and the formation of metabolites at 8 μM Lu AA21004.

Phenotyped human liver microsomes were incubated with 8 μM Lu AA21004 for 60 minutes. The formation of the individual metabolites was correlated to form selective activities. Data shown are the correlation coefficients (r. * p < 0.05 (written in italics). ** p < 0.01 (written in bold). Similar data was obtained following incubation with 50 μM Lu AA21004.

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Sulfoxide</th>
<th>Benzylic alcohol of Lu AA21004</th>
<th>Benzoic acid of Lu AA21004</th>
<th>N-hydroxy-piperazine</th>
<th>4-hydroxy-phenyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>0.5068</td>
<td>0.3190</td>
<td>0.2183</td>
<td>0.3556</td>
<td>0.360</td>
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<tr>
<td>CYP2A6</td>
<td>0.8401**</td>
<td>0.1410</td>
<td>0.2004</td>
<td>0.7556**</td>
<td>0.031</td>
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<tr>
<td>CYP2B6</td>
<td>0.8196**</td>
<td>0.3098</td>
<td>0.0718</td>
<td>0.8764**</td>
<td>0.152</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.4806</td>
<td>0.6110*</td>
<td>0.4080</td>
<td>0.7277**</td>
<td>0.546*</td>
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<tr>
<td>CYP2C19</td>
<td>0.3882</td>
<td>0.1681</td>
<td>0.1547</td>
<td>0.7039**</td>
<td>0.096</td>
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<tr>
<td>CYP2D6</td>
<td>0.1693</td>
<td>0.7639**</td>
<td>0.7445**</td>
<td>0.2024</td>
<td>0.788**</td>
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<tr>
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<td>0.0374</td>
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<tr>
<td>CYP3A1</td>
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<tr>
<td>CYP3A2</td>
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<td>0.3401</td>
<td>0.0464</td>
<td>0.8690**</td>
<td>0.171</td>
</tr>
</tbody>
</table>

¹Dextromethorphan-N-Demethylation, ²Testosterone-6β-hydroxylation

Intercorrelation (R-value): 2A6 and 2B6: 0.8081**, 2A6 and 3A: 0.8460**, 2B6 and 3A: 0.9925**, 2C9 and 4A11: 0.6798**, 2C9 and 3A: 0.7054**, 2C9 and 2C19: 0.6892**, 2C19 and 3A: 0.7243**, 1A2 and 2C19: 0.0884
Table 2. Enzyme kinetic parameters of the metabolism of Lu AA21004 and the benzylic alcohol of Lu AA21004. The assays were performed using pooled human liver microsomes (HLM) or pooled human liver S9 (S9-fraction).

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>Reaction</th>
<th>Km (µM)</th>
<th>Vmax (pmol/min/mg)</th>
<th>Vmax/Km (L/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM</td>
<td>Lu AA21004 → benzylic alcohol</td>
<td>231</td>
<td>262</td>
<td>1.13 x 10^6</td>
</tr>
<tr>
<td></td>
<td>Lu AA21004 → sulfoxide</td>
<td>≥208^1</td>
<td>≥227^1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lu AA21004 → 4-hydroxy-phenyl</td>
<td>323^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lu AA21004 → N-hydroxylated piperazine</td>
<td>300^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>benzylic alcohol → benzoic acid</td>
<td>95</td>
<td>59</td>
<td>0.621 x 10^6</td>
</tr>
<tr>
<td>S9-fraction</td>
<td>benzylic alcohol → benzoic acid</td>
<td>1.7</td>
<td>1567</td>
<td>922 x 10^6</td>
</tr>
</tbody>
</table>

^1Vmax was not reached under the conditions used in this study with substrate concentrations up to 500 µM. The data set was therefore subjected to non-linear regression setting Vmax to ≥ velocity obtained at 500 µM resulting in Km ≥ 208 µM.

^2The Km for the 4-hydroxy-phenyl metabolite and the N-hydroxylated piperazine was estimated using areas obtained by MS/MS due to inadequate sensitivity using radio-detection. The Vmax is therefore expressed in terms of Area/IS/min/mg.
Figure 4

Benzylic alcohol of Lu AA21004

Sulfoxide of Lu AA21004

N-hydroxylated Lu AA21004

4-hydroxy-phenyl Lu AA21004
Figure 5.

A) Benzoic acid of Lu AA21004

B) Benzoic acid of Lu AA21004

Benzylic alcohol of Lu AA21004 (μM)

pmol/min/mg

V (nmol/min/mg)

Benzylic alcohol of Lu AA21004 (μM)