Metabolism of the active metabolite of quetiapine, N-desalkylquetiapine, in vitro

Gry Vibeke Bakken, Espen Molden, Karoline Knutsen, Niclas Lunder and Monica Hermann

Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway (G.V.B., E.M., N.L., M.H.); and Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway (E.M., K.K., M.H.).
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Corresponding author:

Gry Vibeke Bakken

Center for Psychopharmacology, Diakonhjemmet Hospital, P.O. Box 85, Vinderen, N-0319 Oslo, Norway

Telephone: +47 22029940
Fax: +47 22029993

grvb@farmasi.uio.no

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Nonstandard abbreviations:

CLint, intrinsic clearance
ESI, electrospray ionization
HLM, human liver microsomes
P450, cytochrome P450
Abstract

The antipsychotic drug quetiapine has recently been approved for the treatment of uni- and bipolar depression. The antidepressant activity is considered to be mediated by the active metabolite, N-desalkylquetiapine, which is mainly formed by CYP3A4. Little is known about the subsequent elimination of this metabolite. Therefore, this study investigated the possible involvement of cytochrome P450 enzymes in the metabolism of N-desalkylquetiapine. Screening for and interpretation of metabolites were performed by incubating N-desalkylquetiapine in human liver microsomes (HLM) followed by liquid chromatography-tandem mass spectrometry. The possible involvement of CYP enzymes in N-desalkylquetiapine metabolism was evaluated by co-incubation of selective CYP inhibitors in HLM and subsequent experiments with recombinant human CYP enzymes. In HLM experiments, three chromatographic peaks were interpreted as possible metabolites of N-desalkylquetiapine; i.e. N-desalkylquetiapine sulfoxide, 7-hydroxy-N-desalkylquetiapine and an unrecognized metabolite (denoted M3). Inhibition of CYP2D6 (by quinidine) reduced formation of 7-hydroxy-N-desalkylquetiapine by 81%, while the CYP3A4 inhibitor ketoconazole inhibited formation of N-desalkylquetiapine sulfoxide and M3 by 65% and 34%, respectively. Inhibitors of CYP1A2, CYP2C9 and CYP2C19 showed only limited changes in metabolite formation. In recombinant systems, 7-hydroxy-N-desalkylquetiapine was exclusively formed by CYP2D6, whereas N-desalkylquetiapine sulfoxide and M3 were formed by both CYP3A4 and CYP2D6. Overall, intrinsic clearance of N-desalkylquetiapine was 12-fold higher by recombinant CYP2D6 relative to CYP3A4. In conclusion, N-desalkylquetiapine is metabolized by both CYP2D6 and CYP3A4 in vitro with preference for the former enzyme. The pharmacologically active metabolite, 7-hydroxy-N-desalkylquetiapine, was exclusively formed by CYP2D6, whereas the two other metabolites were mainly formed by CYP3A4.
Introduction

Quetiapine is a dibenzothiazepine derivative that belongs to the class of atypical antipsychotics. It is indicated for the treatment of schizophrenia, acute episodes of mania and depression associated with bipolar disorder, and maintenance treatment of bipolar disorder. Additionally, quetiapine was recently approved as an adjunctive treatment of major depressive disorder.

Quetiapine is extensively metabolized by sulfoxidation, hydroxylation, N- and O-dealkylation and oxidation to carboxylic acid (Grimm et al., 1997). Cytochrome P450 3A (CYP3A) is the main enzyme involved in the metabolism of quetiapine, responsible for the sulfoxidation, N- and O-dealkylation, and partially the 7-hydroxylation (Grimm et al., 1997; Bakken et al., 2009). The 7-hydroxy pathway is also catalysed by CYP2D6 (Grimm et al., 2006), but this enzyme is regarded as of minor importance for the overall clearance of quetiapine (Hasselstrøm and Linnet, 2006). N-Desalkyquetiapine is the main active metabolite of quetiapine and is suggested to be responsible for the antidepressant effect (Jensen et al., 2008). The antidepressant activity of N-desalkyquetiapine could be explained by its properties as a potent noradrenaline reuptake inhibitor and a partial agonist at the 5-hydroxytryptamine 1A receptor (Jensen et al., 2008). In addition, N-desalkyquetiapine has demonstrated antidepressant activity in a mouse model (Jensen et al., 2008).

Systemic exposure of N-desalkyquetiapine is reported to be greater than quetiapine at steady-state in psychiatric patients (Bakken et al., 2011), and both N-desalkyquetiapine and the parent drug display an extensive interindividual variability in serum concentration at similar quetiapine dosage (Bakken et al., 2011). N-Desalkyquetiapine is mainly formed by CYP3A4 (Grimm et al., 1997; 2006), but to our knowledge no data on the further metabolism of N-desalkyquetiapine has been published. The aim of this *in vitro* study was therefore to
investigate the possible involvement of cytochrome P450 enzymes in the metabolism of N-desalkylquetiapine.
Materials and methods

Materials

N-Desalkylquetiapine (11-(1-Piperazinyl)-dibenzo[3b,f][1,4]thiazepine, 98% purity) and (+)-N-3-benzynirvanol were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada), while promazine, ketoconazole, quinidine, fluvoxamine and sulfaphenazole were obtained from Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes (from 22 donors of mixed gender) were purchased from BD gentest (Woburn, MA). Microsomes prepared from baculovirus-infected insect cells expressing human CYP2D6 and cytochrome P450 reductase (Supersomes) were also purchased from BD Gentest, whereas similar microsomes expressing CYP3A4 (Xenosomes) were obtained from Larodan Fine Chemicals AB (Malmö, Sweden).

General incubation conditions

All incubations were performed in an incubation matrix consisting of Tris-H$_2$SO$_4$ (118 mM, pH 7.5), MgSO$_4$ (0.5 mM) and NADPH (1.6 mM). Methanol stock solutions of N-desalkylquetiapine were evaporated to dryness and re-dissolved in the incubation matrix. Microsomes were diluted by a solution (pH 7.4) consisting of 0.25 M sucrose, 10 mM Hepes and 2 mM EDTA. The final protein concentration was 0.25 mg/ml in the HLM incubations, and the enzyme concentration was 4 pmol/ml in the incubations performed with recombinant P450 enzymes. Total incubation volume was 220 µl, and the enzyme content was 0.9 pmol in the recombinant systems. All incubations were performed in a shaking water bath at 37 °C, and the reactions were initiated by adding preheated microsomes. Reactions were terminated by adding a precipitation solution consisting of ice-cold acetonitrile and promazine (internal standard, final concentration 4 µM) to the incubation mixture (0.7:1). The samples were kept on ice for at least 30 min before centrifugation at 1600 g for 10 min at 4 °C (Universal 32R,
Hettich Zentrifugen, Tuttlingen, Germany), and the supernatant was centrifuged further at 20,000 g for 10 min (4 °C). Negative controls, consisting of incubation matrix without substrate, were incubated under identical conditions in each experiment.

**Enzyme kinetic studies with HLM**

Enzyme kinetics was studied by measuring metabolite formation in incubations with *N*-desalkylquetiapine concentrations ranging from 1 µM to 1000 µM. The samples were incubated for 10 min, and the general conditions were followed. Preliminary studies were performed to test the linearity of the incubation assay with regard to protein concentration and incubation time. Linearity was confirmed for all metabolites for up to 30 min incubation time and up to 0.25 mg/ml protein.

**Incubations with specific CYP inhibitors**

To determine CYP enzymes involved in the metabolism of *N*-desalkylquetiapine, HLM were incubated in the presence of CYP inhibitors. The inhibitors used were fluvoxamine (5 µM), sulfaphenazole (10 µM), benzylmirvanol (10 µM), quinidine (5 µM) and ketoconazole (1 µM), which inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, respectively. Methanol stock solutions of the inhibitors were diluted with the incubation matrix and preincubated with the substrate. Control samples without an inhibitor were prepared correspondingly. Final methanol concentration was 0.9% both in the samples with inhibitors and the control samples. All samples were incubated for 30 min, and the incubations were performed in duplicate. The concentration of *N*-desalkylquetiapine was 3 µM. The peak area ratios (metabolite/internal standard) in the control samples were defined as 100%, and the peak area ratios in the incubations with inhibitors were expressed as a percentage of the formation in the control samples.
Incubations with recombinant CYP enzymes

Based on the HLM experiment with inhibitors, CYP enzymes shown to be involved in the metabolism of \(N\)-desalkylquetiapine were further examined using recombinant systems. Substrate depletion was measured in the time interval from 0 to 60 min. The initial concentration of \(N\)-desalkylquetiapine in these experiments was 3 \(\mu\)M, i.e. well below the \(K_m\) values for the formation of all metabolites (initially determined in the enzyme kinetic studies with HLM). Additionally, metabolite formation was studied after 60 min incubation. For each recombinant system studied, three separate experiments were performed. All incubations in each experiment were performed in duplicate.

Metabolite identification

Screening for metabolites was performed by incubation of 50 \(\mu\)M \(N\)-desalkylquetiapine with HLM for 30-90 min and the general incubation conditions described above were followed. The samples were subjected to the following MS scans using a Quattro Micro tandem mass spectrometer (Waters, Milford, MA): full scan, single ion recording, daughter ions scan, parent scan, neutral loss and multiple reaction monitoring. Electrospray ionization (ESI) was performed in the positive mode. Capillary voltage was 0.9 kV, source temperature and desolvation temperature was 100 and 450 °C, respectively, and desolvation gas flow was 450 L/h. The cone and collision voltages were varied between 15-40 V and 20-30 eV, respectively. Incubations with increasing incubation time and increasing microsomal protein concentration were performed to confirm that the detected peaks were metabolites. Interpretation of possible metabolites was performed by studying the fragmentation pattern. During the initial HLM experiments, the formation of three metabolites of \(N\)-desalkylquetiapine was suspected (referred to as M1, M2 and M3 in the further text).
Analytical assay

Pre-treated samples were analyzed using an Acquity ultra performance liquid chromatograph (Waters) with the tandem mass spectrometer described above operated in ESI-positive mode. Injection volume was 5 µl and chromatographic separation was performed on a Waters BEH Shield RP18 column (1.7 µm, 1.0 x 100 mm). The analytes were eluted at a flow rate of 0.2 ml/min by a mobile phase gradient from 10 to 90% acetonitrile in ammonium acetate (10 mM, pH 4.8) within 8 min. Total run time was 10 min. The retention times were 4.0 min for M1, 4.3 min for M2, 6.2 min for N-desalkylquetiapine, 6.5 min for promazine and 7.2 min for M3. Cone voltages were 30, 30, 40, 35 and 30 V, and collision energies were 20, 30, 20, 20 and 20 eV for N-desalkylquetiapine, M1, M2, M3 and promazine, respectively. Detection was performed in the multiple reaction mode at the following transitions: \( m/z \ 296 \rightarrow 210 \) for N-desalkylquetiapine, \( m/z \ 312 \rightarrow 221 \) for M1, \( m/z \ 312 \rightarrow 269 \) for M2, \( m/z \ 312 \rightarrow 210 \) for M3 and \( m/z \ 285 \rightarrow 212 \) for promazine. N-Desalkylquetiapine was quantified using quadratic standard curves with a 1/Y weighting. As reference standards for the metabolites were not available, the metabolites were quantified using peak area ratio (metabolite/internal standard).

Data analysis

Substrate depletion data were fitted to the monoexponential decay model (eq. 1):

\[
S_t = S_0 e^{-kt}
\]  

(1)

where \( S_t \) is the substrate concentration at time \( t \), \( S_0 \) is the initial substrate concentration and \( k \) is the rate constant (min\(^{-1}\)). Visual inspection of log-transformed substrate depletion curves was used to decide the time interval where the depletion of N-desalkylquetiapine followed monoexponential decay (data not shown). In each separate experiment, \( k \) was estimated and the intrinsic clearance (CL\(_{int} \)) was calculated (eq. 2):
\[
\text{CL}_{\text{int}} = kV
\]  
(2)

where \( V \) is the incubation volume. \( \text{CL}_{\text{int}} \) was then divided by the CYP2D6 or CYP3A4 enzyme content (pmol) in the incubation mixture.

Data from studies with increasing \( N \)-desalkylquetiapine concentrations in HLM were fitted to different enzyme kinetic models, i.e. the classical Michaelis Menten model (eq. 3), substrate activation (eq. 4), substrate inhibition (eq. 5) and the two-enzyme Michaelis Menten model (eq. 6) by nonlinear regression (Houston and Kenworthy, 2000; Venkatakrishnan et al., 2001). The choice of the best-fit kinetic model was based on visual inspection of plots with the fitted functions and Eadie-Hofstee plots (plots not shown), in addition to calculations based on the Akaike information criterion (Venkatakrishnan et al., 2001). Enzyme kinetic parameters for metabolite formation were then estimated. The equations for the kinetic models used were:

\[
\nu = \frac{V_{\max} S}{K_m + S}
\]  
(3)

\[
\nu = \frac{V_{\max} S^n}{S^{50}_n + S^n}
\]  
(4)

\[
\nu = \frac{V_{\max}}{1 + (K_m / S) + (S / K_s)}
\]  
(5)

\[
\nu = \frac{V_{\max 1} S}{K_{m1} + S} + \frac{V_{\max 2} S}{K_{m2} + S}
\]  
(6)

where \( \nu \) is the rate of metabolite formation, \( V_{\max} \) is the maximum rate of metabolite formation, \( K_m \) is the Michaelis-Menten constant, \( S^{50}_n \) is the substrate concentration at half the maximal rate, \( n \) is the Hill coefficient, \( K_s \) is the substrate inhibition constant. \( K_{m1}/V_{\max 1} \) and \( K_{m2}/V_{\max 2} \) are the corresponding values (as mentioned above) for the reaction catalyzed by enzyme 1 and 2, respectively.
Nonlinear regression and estimation of kinetic parameters was performed using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA).
Results

Metabolite identification

Four chromatographic peaks representing possible metabolites were detected in positive ESI after incubation of N-desalkylquetiapine with HLM, all with \textit{m/z} 312 as the molecular ion (retention times 4.0, 4.1, 4.3 and 7.2 min; Fig. 1). All four peaks increased with increasing incubation time and microsomal protein concentration (data not shown).

The two peaks with retention time around 4 min were not baseline separated. After studying the daughter ion mass spectra of the two peaks, a similar fragmentation pattern was found for both peaks. The two peaks were therefore interpreted to be isomeric forms of the same metabolite (M1, Fig. 1) and the formation of this metabolite was calculated as the sum of the two peaks. Accordingly, formation of three metabolites (M1, M2 and M3) from \textit{N}-desalkylquetiapine was interpreted after the incubations with HLM (Fig. 1).

The daughter ion mass spectra (Fig. 2), and suggested fragmentation patterns of two proposed metabolites (table 1), indicated that the M1 and M2 peaks represented the sulfoxide and 7-hydroxy metabolites of \textit{N}-desalkylquetiapine, respectively. The main daughter ions formed by fragmentation of M1 were \textit{m/z} 264 and 221; these fragment ions are possibly formed by loss of a sulfoxide-group and loss of a sulfoxide-group concurrent with piperazine ring cleavage, respectively (table 1). The fragment ion \textit{m/z} 221 was also the main fragment seen in the daughter ion mass spectrum of the sulfoxide metabolite of quetiapine (Hasselstrøm and Linnet, 2006). Therefore, M1 most likely corresponds to the sulfoxide metabolite of \textit{N}-desalkylquetiapine. For M2, the main daughter ions were \textit{m/z} 269 and 226, which could be formed by piperazine ring cleavage and loss of the piperazine group, respectively. Furthermore, the fragment ion \textit{m/z} 269 was the main daughter ion of the 7-hydroxy metabolite of quetiapine (Hasselstrøm and Linnet, 2006). These findings imply that M2 is 7-hydroxy-\textit{N}-
desalkylquetiapine. Consequently, M1 is hereafter denominated \(N\)-desalkylquetiapine sulfoxide and M2 is denominated 7-hydroxy-\(N\)-desalkylquetiapine.

The identity of the peak M3 could not be unambiguously determined. Two of the main fragments, i.e. \(m/z\) 210 and 253, were analogous to the main daughter fragments of \(N\)-desalkylquetiapine (spectrum not shown). This could imply that the oxidation has not occurred within the dibenzothiazepine ring structure as with \(N\)-desalkylquetiapine sulfoxide and 7-hydroxy-\(N\)-desalkylquetiapine, but some of the other fragment ions are identical with these two metabolites.

The metabolites were negligible in negative controls without substrate. In HLM experiments with 3 \(\mu\)M \(N\)-desalkylquetiapine incubated for 60 min, the peak area ratio of all metabolites were <0.5% in the negative controls compared to samples containing substrate.

**Enzyme kinetic studies with HLM**

Kinetic studies using increasing concentrations of \(N\)-desalkylquetiapine showed that the formation of M3 followed classical Michaelis Menten kinetics, whereas the formation of 7-hydroxy-\(N\)-desalkylquetiapine and \(N\)-desalkylquetiapine sulfoxide was best described by the substrate inhibition model and the two-enzyme Michaelis Menten model, respectively (Fig. 3). The coefficient of determination (R\(^2\)) for the nonlinear regression was >0.98 for all metabolites. The estimated \(K_m\) value for the formation of M3 was 199 \(\mu\)M (95% CI, 175-222 \(\mu\)M), whereas the \(K_{m1}\) and \(K_{m2}\) value for the formation of \(N\)-desalkylquetiapine sulfoxide was 15 \(\mu\)M (95% CI, 4-26 \(\mu\)M) and 459 \(\mu\)M (95% CI, 181-738 \(\mu\)M), respectively. For the formation of 7-hydroxy-\(N\)-desalkylquetiapine the \(K_m\) value was 110 \(\mu\)M (95% CI, 62-157 \(\mu\)M), and the \(K_{si}\) value was calculated to 824 \(\mu\)M (95% CI, 357-1291 \(\mu\)M). As authentic standards for the metabolites were not available, the maximum reaction rate (\(V_{max}\)) could not be determined.
HLM studies with CYP inhibitors

Effect of chemical inhibitors on the formation of the metabolites of N-desalkylquetiapine was studied in HLM (Fig. 4). Co-incubation with quinidine (5 µM) inhibited formation of 7-hydroxy-N-desalkylquetiapine by 81%, while ketoconazole (1 µM) inhibited formation of N-desalkylquetiapine sulfoxide and M3 by 65% and 34%, respectively. Fluvoxamine inhibited formation of 7-hydroxy-N-desalkylquetiapine by approximately 20%. Benzylnivanol and sulfaphenazole did not inhibit formation of any of the metabolites in the tested concentrations.

Studies with recombinant CYP enzymes

The inhibition studies in HLM revealed that CYP2D6 (quinidine inhibition) and CYP3A4 (ketoconazole inhibition) were involved in the metabolism of N-desalkylquetiapine to a considerable extent. Therefore, metabolism of N-desalkylquetiapine was further studied in recombinant CYP2D6 and CYP3A4 microsomes. Substrate depletion of N-desalkylquetiapine (3 µM) by CYP2D6 and CYP3A4 showed monoexponential decay within the time range 0-60 min (Fig. 5), and the $R^2$ values for the curve fit were >0.9 for CYP2D6 and >0.2 for CYP3A4. The substrate depletion by CYP2D6 and CYP3A4 was about 47% and 6%, respectively, after 60 minutes of incubation. CL_int of N-desalkylquetiapine was calculated to be 3.0 ± 0.3 µl/min/pmol P450 (mean ± S.E.M.) via CYP2D6 and 0.25 ± 0.08 µl/min/pmol P450 via CYP3A4.

Measurement of N-desalkylquetiapine metabolites after 60 min incubation in CYP2D6 and CYP3A4 microsomes revealed that the two enzymes were involved to different extents in the formation of the three metabolites. 7-Hydroxy-N-desalkylquetiapine was almost exclusively formed by CYP2D6, whereas CYP2D6 and CYP3A4 were to the same degree involved in the formation of N-desalkylquetiapine sulfoxide. M3 was formed by both
CYP2D6 and CYP3A4, but the formation was 1.6-fold higher by CYP3A4 relative to CYP2D6.
Discussion

The present study demonstrates that the metabolism of N-desalkylquetiapine \textit{in vitro} is mainly mediated by CYP2D6 and to a lesser extent by CYP3A4. Based on CL$_{\text{int}}$ estimations in recombinant systems, CYP2D6 was 12-fold more effective in catalyzing metabolism of N-desalkylquetiapine than CYP3A4. However, as the expression of CYP3A4 protein has been reported to be approximately 15-fold higher compared to CYP2D6 in human liver (Shimada et al., 1994), one might expect that the relative importance of these two enzymes in the \textit{in vivo} CL$_{\text{int}}$ of N-desalkylquetiapine is fairly the same.

Three metabolites of N-desalkylquetiapine were detected, two of which were interpreted as N-desalkylquetiapine sulfoxide and 7-hydroxy-N-desalkylquetiapine (Fig. 6). CYP2D6 and CYP3A4 were to a different extent involved in the formation of the metabolites. Quinidine, a selective CYP2D6 inhibitor strongly inhibited the formation of 7-hydroxy-N-desalkylquetiapine in HLM, and this metabolite was also exclusively formed by CYP2D6 in the recombinant systems employed. Hence, these results suggest that CYP2D6 is the main enzyme involved in the formation of 7-hydroxy-N-desalkylquetiapine. This metabolite is considered pharmacologically active and the plasma concentration 12 hours post-dose has been reported to be about 18\% of the plasma concentration of quetiapine (Gefvert et al., 1998). Grimm \textit{et al.} have previously shown that CYP2D6 is involved in the 7-hydroxylation of the parent drug quetiapine (Grimm et al., 2006). However, while CYP2D6-mediated 7-hydroxylation was a major metabolic pathway of N-desalkylquetiapine \textit{in vitro}, quetiapine has \textit{in vitro} showed a limited preference for CYP2D6 (Hasselstrøm and Linnet, 2006). These findings might indicate that individual variability in CYP2D6 metabolism is more relevant for the active metabolite N-desalkylquetiapine than the parent drug.

Formation of 7-hydroxy-N-desalkylquetiapine was also inhibited by approximately 20\% in presence of fluvoxamine. Fluvoxamine is a potent inhibitor of CYP1A2 (Brosen et al.,
1993), but has also demonstrated inhibitory effect on CYP2D6, with reported $K_i$ values of 8 and 17 µM versus sparteine oxidation and desipramine hydroxylation, respectively (Crewe et al., 1992; von Moltke et al., 1995). Therefore, the observed reduction in the formation of 7-hydroxy-$N$-desalkylquetiapine by fluvoxamine could be caused by an inhibitory effect on CYP1A2 or CYP2D6.

The formation of $N$-desalkylquetiapine sulfoxide and M3 in HLM was only inhibited by the selective CYP3A4 inhibitor ketoconazole. However, ketoconazole inhibited the formation of M3 with only 34%, suggesting that other enzymes besides those examined in the present study could be involved in the formation of this metabolite. In recombinant systems, both CYP2D6 and CYP3A4 were involved in the formation of $N$-desalkylquetiapine sulfoxide and M3. Considering the substantially higher content of CYP3A4 protein compared to CYP2D6 in human liver, these findings suggest that CYP3A4 is of greater importance for the formation of these metabolites than CYP2D6. This is in accordance with previous studies reporting that CYP3A4 is the major enzyme implicated in the sulfoxidation pathway of quetiapine (Grimm et al., 2006).

In the HLM studies with CYP inhibitors, methanol (0.9%) was used to ensure solubility of the inhibitors. Busby et al. have reported that 1% methanol has inhibited the activity of CYP1A2, CYP2C9, CYP2C19 and CYP3A4 by ≤12%, whereas CYP2D6 activity was inhibited by 26% (Busby et al., 1999). This suggests that the effect of the CYP2D6 inhibitor quinidine on formation of 7-hydroxy-$N$-desalkylquetiapine could have been underestimated and could also explain that quinidine had no effect on the formation of $N$-desalkylquetiapine sulfoxide and M3, while both metabolites were formed by recombinant CYP2D6. Hence, the initial screening experiments in HLM using 0.9% methanol as cosolvent could not be applied for quantitative comparisons of the contribution of various CYP enzymes in the metabolism of $N$-desalkylquetiapine.
In the experiments performed with recombinant systems, both the CYP3A4 and CYP2D6 microsomes were coexpressed with cytochrome P450 reductase, but not with cytochrome b5. Cytochrome b5 has shown to enhance the metabolism of several substrates via CYP3A4 (Yamazaki et al., 1996; Klees et al., 2005). On the contrary, no effect of cytochrome b5 on CYP2D6 activity has been demonstrated (Yamazaki et al., 1997; 2002). Thus, the calculated CL_{int} of N-desalkylquetiapine by CYP3A4 in the present study could potentially have been higher in microsomes coexpressed with cytochrome b5. However, the effect of cytochrome b5 on CYP3A4 activity is substrate specific (Yamaori et al., 2003) and recently we showed that the in vitro metabolism of quetiapine was actually lower in recombinant CYP3A4 coexpressed with cytochrome b5 (Bakken et al., 2009). The CL_{int} value for CYP3A4-mediated metabolism is also more uncertain compared to CYP2D6 as the substrate loss in CYP3A4 microsomes was less than recommended (Jones and Houston, 2004). Furthermore, involvement of other enzymes than CYP3A4 and CYP2D6 in the elimination of N-desalkylquetiapine besides those examined could not be ruled out. Consequently, in vivo studies are necessary to determine the quantitative involvement of CYP3A4 and CYP2D6 in the metabolism of N-desalkylquetiapine.

We have previously reported a fivefold difference in dose-adjusted serum concentrations of N-desalkylquetiapine in psychiatric patients (Bakken et al., 2011). This could be due to both variability in the formation and subsequent elimination of N-desalkylquetiapine. CYP3A4 is the main enzyme responsible for the formation of N-desalkylquetiapine (Grimm et al., 1997; 2006), and the present study demonstrated that CYP3A4 and CYP2D6 are the main CYP enzymes involved in the subsequent metabolism of N-desalkylquetiapine. Individual variability in the enzyme activity of both CYP3A4 and CYP2D6 is extensive and could be of importance for the pharmacokinetic variability of N-desalkylquetiapine. Genetic polymorphism in the expression of CYP2D6 is well known.
(Zhou, 2009) and may potentially be associated to the interindividual variability in serum concentration of N-desalkylquetiapine. Since N-desalkylquetiapine is suggested to be responsible for the antidepressant activity of quetiapine (Jensen et al., 2008), it is important to follow up the present in vitro findings with in vivo studies to elucidate whether genetic polymorphism in CYP2D6 is of clinical importance during treatment with quetiapine. This will also provide conclusive evidence regarding the potential difference in impact of CYP2D6-mediated metabolism for the overall clearance of the active metabolite compared to the parent drug.

In conclusion, N-desalkylquetiapine is mainly metabolized by CYP2D6 and CYP3A4 in vitro. Three metabolites were detected, two of which were interpreted as N-desalkylquetiapine sulfoxide and 7-hydroxy-N-desalkylquetiapine. CYP2D6 and CYP3A4 were to different extents involved in the formation of the metabolites. The active metabolite, 7-hydroxy-N-desalkylquetiapine, was exclusively formed by CYP2D6, whereas the two other metabolites were mainly formed by CYP3A4. Involvement of the polymorphic enzyme CYP2D6 in the metabolism of N-desalkylquetiapine could be of clinical importance during quetiapine treatment and might be a factor contributing to the extensive interindividual variability in serum concentration of N-desalkylquetiapine.
Authorship contributions

Participated in research design: Bakken, Molden, Knutsen, and Hermann

Conducted experiments: Bakken and Knutsen

Contributed new reagents or analytic tools: Bakken and Lunder

Performed data analysis: Bakken and Knutsen

Wrote or contributed to the writing of the manuscript: Bakken, Molden, Knutsen, Lunder, and Hermann
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**Figure legends**

FIG. 1. Ion chromatograms of \( N \)-desalkylquetiapine (NQ), promazine (internal standard, IS) and metabolites M1-M3 in HLM incubations.

FIG. 2. Daughter ion spectra of the metabolites M1-M3 in HLM incubations.

FIG. 3. Formation of \( N \)-desalkylquetiapine sulfoxide (NQ-S), 7-hydroxy-\( N \)-desalkylquetiapine (NQ-7H) and M3 in HLM incubations with \( N \)-desalkylquetiapine concentrations ranging from 1-1000 µM. Data for all metabolites are arbitrary (peak area ratio) and were fitted to enzyme kinetic models using nonlinear regression. The formation of M3 followed classical Michaelis Menten kinetics (eq. 3), whereas the formation of 7-hydroxy-\( N \)-desalkylquetiapine and \( N \)-desalkylquetiapine sulfoxide were best described by the substrate inhibition model (eq. 5) and the two-enzyme Michaelis Menten model (eq. 6), respectively. Data from one experiment are presented.

FIG. 4. Percent formation of \( N \)-desalkylquetiapine sulfoxide (NQ-S), 7-hydroxy-\( N \)-desalkylquetiapine (NQ-7H) and M3 in HLM incubated with the CYP inhibitors benzynirvanol (CYP2C19), fluvoxamine (CYP1A2), ketoconazole (CYP3A4), quinidine (CYP2D6) and sulfaphenazole (CYP2C9) compared to control samples without inhibitors. The bars represent mean of duplicate incubations. The concentration of \( N \)-desalkylquetiapine was 3 µM and all samples were incubated for 30 min.

FIG. 5. Substrate depletion of \( N \)-desalkylquetiapine (3 µM) in recombinant CYP2D6 and CYP3A4 microsomes. Data are presented as mean ± S.E.M. (n=3).
FIG. 6. Metabolic pathways of N-desalkylquetiapine *in vitro*
### Tables

#### TABLE 1

Possible daughter ions ($m/z$) of two proposed metabolites of $N$-desalkylquetiapine, $N$-desalkylquetiapine sulfoxide and 7-hydroxy-$N$-desalkylquetiapine, derived from bond cleavages (a, b and c)

<table>
<thead>
<tr>
<th></th>
<th>Sulfoxide metabolite (M1)</th>
<th>7-Hydroxy-metabolite (M2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_1 = H$  $R_2 = O$</td>
<td>$R_1 = OH$  $R_2 = \text{none}$</td>
</tr>
<tr>
<td>$m/z$</td>
<td>$269$</td>
<td>$269$</td>
</tr>
<tr>
<td>$m/z$</td>
<td>$226$</td>
<td>$226$</td>
</tr>
<tr>
<td>$a$</td>
<td>$264$</td>
<td>-</td>
</tr>
<tr>
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<td>$221$</td>
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</tbody>
</table>

![Diagram of metabolite structures]
Figure 1
Figure 3
Figure 5
Figure 6

N-Desalkylquetiapine

N-Desalkylquetiapine sulfoxide

CYP3A4

CYP2D6

7-Hydroxy-N-desalkylquetiapine

N-Desalkylquetiapine

CYP3A4

M3