Metabolism of the Active Metabolite of Quetiapine, N-Desalkylquetiapine In Vitro

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ABSTRACT:
The antipsychotic drug quetiapine has been approved for the treatment of unipolar 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 (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 P450 enzymes in N-desalkylquetiapine metabolism was evaluated by coincubation of selective P450 inhibitors in HLM and subsequent experiments with recombinant human P450 enzymes. In HLM experiments, three chromatographic peaks were interpreted as possible metabolites of N-desalkylquetiapine, namely, 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%, whereas 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. In addition, quetiapine was 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). 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 catalyzed by CYP2D6 (Grimm et al., 2006), but this enzyme is of minor importance for the overall clearance of quetiapine (Hasselstrøm and Linnet, 2006). N-Desalkylquetiapine 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-desalkylquetiapine 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-desalkylquetiapine has demonstrated antidepressant activity in a mouse model (Jensen et al., 2008).

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

Materials and Methods
Materials. N-Desalkylquetiapine (11-(1-piperazinyl)-dibenzo[b,][1,4]thiazepine, 98% purity) and (+)-N-3-benzylnirvanol were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada), and promazine, ketoconazole, quinidine, fluvoxamine, and sulfaphenazole were obtained from Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes (HLM) (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 were used as recombinant CYP2D6.

Abbreviations. P450, cytochrome P450; M, metabolite; CLint, intrinsic clearance; HLM, human liver microsomes; CI, confidence interval; IS, internal standard.

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experiments, the formation of three metabolites of N-desalkylquetiapine was suspected (referred to as M1, M2, and M3 in the further text).

Analytical Assay. Pretreated samples were analyzed using an ACQUITY ultraperformance liquid chromatograph (Waters) with the tandem mass spectrometer described in the previous section operated in electrospray ionization—positive mode. Injection volume was 5 μl, and chromatographic separation was performed on a Waters BEH Shield RP18 column (1.7 μm, 1.0 × 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 for M1, 4.3 for M2, 6.2 for N-desalkylquetiapine, 6.5 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 → 210 for N-desalkylquetiapine, m/z 312 → 221 for M1, m/z 312 → 269 for M2, m/z 312 → 210 for M3, and m/z 285 → 212 for promazine. N-Desalkylquetiapine was quantified using quadratic standard curves with a 1/Y weighting. Because reference standards for the metabolites were not available, the metabolites were quantified using peak area ratio (metabolite/IS).

Data Analysis. Substrate depletion data were fitted to the monoeXponential decay model (eq. 1):

$$S_t = S_0 e^{-kt}$$  \hspace{1cm} (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):

$$CL_{int} = \frac{kV}{W}$$  \hspace{1cm} (2)

where $V$ is the incubation volume. $CL_{int}$ 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 classic 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; Venkatarkrishnan 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 (Venkatarkrishnan et al., 2001). Enzyme kinetic parameters for metabolite formation were then estimated. The equations for the kinetic models used were as follows:

$$v = \frac{V_{max}S}{K_m + S}$$  \hspace{1cm} (3)

$$v = \frac{V_{max}S}{S_0 + S^*}$$  \hspace{1cm} (4)

$$v = \frac{V_{max}}{(1 + (K_{si} / S) + (S / K_m))}$$  \hspace{1cm} (5)

$$v = \frac{V_{max1}S}{K_m + S} + \frac{V_{max2}S}{K_m + S}$$  \hspace{1cm} (6)

where $v$ is the rate of metabolite formation, $V_{max}$ is the maximal rate of metabolite formation, $K_m$ is the Michaelis-Menten constant, $S_{si}$ is the substrate concentration at half the maximal rate, $n$ is the Hill coefficient, and $K_{si}$ is the substrate inhibition constant. $K_m$ and $V_{max1}$ correspond to the high-affinity/low-capacity site, and $K_m$ and $V_{max2}$ correspond to the low-affinity/high-capacity site.

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 electrospray ionization after incubation of N-desalkylquetiapine with HLM, all with 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. Therefore, the two peaks were 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. Consequently, formation of three metabolites (M1, M2, and M3) from 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 N-desalkylquetiapine, respectively. The main daughter ions formed by fragmentation of M1 were m/z 264 and m/z 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 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 N-desalkylquetiapine. For M2, the main daughter ions were m/z 269 and m/z 226, which could be formed by piperazine ring cleavage and loss of the piperazine group, respectively. Furthermore, the fragment ion 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-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 m/z 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 to these two metabolites.

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

Enzyme Kinetic Studies with HLM. Kinetic studies using increasing concentrations of N-desalkylquetiapine showed that the formation of M3 followed classic 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 (R²) for the nonlinear regression was > 0.98 for all metabolites. The estimated Kₘ value for the formation of M3 was 199 μM [95% confidence interval (CI), 175–222 μM], whereas the Kₘ₁ and Kₘ₂ values for the formation of N-desalkylquetiapine sulfoxide were 15 (95% CI, 4–26 μM) and 459 μM (95% CI, 181–738 μM), respectively. For the formation of 7-hydroxy-N-desalkylquetiapine, the Kₘ value was 110 μM (95% CI, 62–157 μM), and the Kₛ value was calculated to 824 μM (95% CI, 351–1291 μM). Because authentic standards for the metabolites were

![](https://example.com/image1.png)

Fig. 1. Ion chromatograms of N-desalkylquetiapine (NQ), promazine (IS), and metabolites M1 to M3 in HLM incubations.
not available, the maximal reaction rate ($V_{\text{max}}$) could not be determined.

**HLM Studies with P450 Inhibitors.** Effect of chemical inhibitors on the formation of the metabolites of N-desalkylquetiapine was studied in HLM (Fig. 4). Coincubation with quinidine (5 $\mu$M) inhibited formation of 7-hydroxy-N-desalkylquetiapine by 81%, whereas ketoconazole (1 $\mu$M) inhibited formation of N-desalkylquetiapine sulfoxide and M3 by 65 and 34%, respectively. Fluvoxamine inhibited formation of 7-hydroxy-N-desalkylquetiapine by approximately 20%. Benzylvinanol and sulfaphenazole did not inhibit formation of any of the metabolites in the tested concentrations.

**Studies with Recombinant P450 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 $\mu$M) by CYP2D6 and CYP3A4 showed monoexponential decay within the time range 0 to 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 approximately 47 and 6%, respectively, after 60 min of incubation. CL$_{int}$ of N-desalkylquetiapine was calculated to be 3.0 ± 0.3 (mean ± S.E.M.) via CYP2D6 and 0.25 ± 0.08 $\mu$L · min$^{-1}$ · pmol P450$^{-1}$ via CYP3A4.

Measurement of N-desalkylquetiapine metabolites after 60 min of 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 in vitro is mainly mediated by CYP2D6 and to a lesser extent by CYP3A4.

### 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>Metabolite Structure</th>
<th>Bond Cleavage</th>
<th>Sulfoxide Metabolite (M1) $R_1 = H$</th>
<th>7-Hydroxy-metabolite (M2) $R_1 = OH$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_2 = O$</td>
<td>m/z</td>
<td>m/z</td>
</tr>
<tr>
<td>a</td>
<td></td>
<td>269</td>
<td>269</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td>226</td>
<td>226</td>
</tr>
<tr>
<td>c</td>
<td></td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>a + c</td>
<td></td>
<td>221</td>
<td></td>
</tr>
</tbody>
</table>
extent by CYP3A4. On the basis of CLint estimations in recombinant systems, CYP2D6 was 12-fold more effective in catalyzing metabolism of N-desalkylquetiapine than CYP3A4. However, because the expression of CYP3A4 protein has been reported to be approximately 15-fold higher compared with CYP2D6 in human liver (Shimada et al., 1994), one might expect that the relative importance of these two enzymes in the in vivo CLint 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 and N-desalkylquetiapine sulfoxide was 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.

Formation of 7-hydroxy-N-desalkylquetiapine was also inhibited by approximately 20% in the presence of fluvoxamine. Fluvoxamine is a potent inhibitor of CYP1A2 (Brøsen et al., 1993) but has also demonstrated inhibitory effect on CYP2D6, with reported Kᵢ 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 inhibited only 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 with 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 P450 inhibitors, methanol (0.9%) was used to ensure solubility of the inhibitors. Busby et al. (1999) have reported that 1% methanol has inhibited the activity of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 by ≤12%, whereas CYP2D6 activity was inhibited by 26%. 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, whereas 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 P450 enzymes in the metabolism of N-desalkylquetiapine.

In the experiments performed with recombinant systems, both the CYP3A4 and the 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 CLint 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 we have previously shown that the in vitro metabolism of quetiapine was actually lower in recombinant CYP3A4 coexpressed with cytochrome b₅ (Bakken et al., 2009). The CLint value for CYP3A4-mediated metabolism is also more uncertain compared with CYP2D6, because the substrate loss in CYP3A4 microsomes was less than recommended (Jones and Houstoun, 2004). Furthermore, involvement of other enzymes than CYP3A4 and CYP2D6 in the elimination of N-desalkylquetiapine besides those examined cannot 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 5-fold difference in dose-adjusted serum concentrations of N-desalkylquetiapine in psychiatric patients (Bakken et al., 2011). This could be due to variability in both the formation and the 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 P450 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. Because 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 with 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.

**References**


Jensen NH, Rodrigut RM, Caron MG, Wetsel WC, Rothman RB, and Roth BL (2008)
N-desalkyl奎乙丙醇，一种强去甲肾上腺素重吸收抑制剂和 partial 5-HT1A agonist，作为奎乙丙醇抗抑郁活性的假定介导物。*Neuropsychopharmacology* 33:2303–2312.


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