

**METABOLISM OF QUETIAPINE BY CYP3A4 AND CYP3A5 IN PRESENCE OR  
ABSENCE OF CYTOCHROME B<sub>5</sub>**

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**Nonstandard abbreviations:**

CL<sub>int</sub>, intrinsic clearance

cyt b<sub>5</sub>, cytochrome b<sub>5</sub>

LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry

### Abstract

The antipsychotic drug quetiapine is extensively metabolised by CYP3A4, but little is known about the possible influence of the polymorphic enzyme CYP3A5. This *in vitro* study investigated the relative importance of CYP3A4 and CYP3A5 in the metabolism of quetiapine and compared the metabolic pattern by the two enzymes, in presence or absence of cytochrome b<sub>5</sub>. CL<sub>int</sub> of quetiapine was determined by the substrate depletion approach in CYP3A4 and CYP3A5 insect cell microsomes with or without coexpressed cytochrome b<sub>5</sub>. Formation of the metabolites quetiapine sulfoxide, N-desalkylquetiapine, O-desalkylquetiapine and 7-hydroxyquetiapine by CYP3A4 and CYP3A5 were compared in the different microsomal preparations. CL<sub>int</sub> of quetiapine by CYP3A5 was less than 35% relative to CYP3A4. CL<sub>int</sub> was higher (3-fold) in CYP3A4 microsomes without cytochrome b<sub>5</sub> compared to CYP3A4 microsomes with coexpressed cytochrome b<sub>5</sub>, whereas in CYP3A5 microsomes CL<sub>int</sub> was similar for both microsomal preparations. Metabolism of quetiapine by CYP3A5 revealed a different metabolic pattern compared to CYP3A4. The results indicated that O-desalkylquetiapine constituted a higher proportion of the formed metabolites by CYP3A5 compared to CYP3A4. In conclusion, the present study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine, regardless of the presence of cytochrome b<sub>5</sub>. However, a different metabolic pattern by CYP3A5 compared to CYP3A4 could possibly result in different pharmacological and/or toxicological effects of quetiapine in patients expressing CYP3A5.

## Introduction

Quetiapine, a dibenzothiazepine derivative, is an atypical antipsychotic used for the treatment of schizophrenia and acute episodes of mania. Recently, the U.S. Food and Drug Administration also approved quetiapine for the treatment of depressive episodes associated with bipolar disorder.

Quetiapine is extensively metabolized in the liver by the cytochrome P450 (CYP) system, primarily by CYP3A (Grimm et al., 1997; Grimm et al., 2006). The major metabolic pathways of quetiapine are through sulfoxidation, N- and O-dealkylation, and, to a lesser degree, through 7-hydroxylation (Grimm et al., 1997) (Fig. 1). The pharmacologically inactive metabolite quetiapine sulfoxide is considered to be the main metabolite of quetiapine (DeVane and Nemeroff, 2001), whereas the most important active metabolite appears to be N-desalkylquetiapine (McIntyre et al., 2007). Studies indicate that N-desalkylquetiapine, unlike the parent compound, is a potent inhibitor of the noradrenergic transporter and has partial agonist activity at the serotonin 5-HT<sub>1A</sub> receptor (Goldstein et al., 2007; Jensen et al., 2008). In addition, N-desalkylquetiapine was found to possess antidepressive-like activity in a mouse model (Jensen et al., 2008). This suggests that the antidepressive activity of quetiapine is, at least partly, mediated by N-desalkylquetiapine. The active metabolite 7-hydroxyquetiapine is formed by CYP2D6 in addition to by CYP3A (Grimm et al., 2006). Because of the low plasma concentration of this metabolite (Gefvert et al., 1998), genetic polymorphism in CYP2D6 is unlikely to be of importance for the *in vivo* metabolism of quetiapine.

CYP3A4 and CYP3A5 comprise the two main CYP3A isoforms in adults. The individual variability in phenotype is substantial for both enzymes, but this variability is linked to genetic polymorphism only for CYP3A5 (Westlind-Johnsson et al., 2003). CYP3A5 is expressed in about 10-30% of Caucasians, 30% of Japanese and 60% of African Americans (Kuehl et al., 2001; Burk and Wojnowski, 2004). The relative contribution of CYP3A5 to

total hepatic CYP3A protein differs, but in some individuals CYP3A5 can constitute more than 50% of total CYP3A (Kuehl et al., 2001; Lin et al., 2002; Westlind-Johnsson et al., 2003). Individuals expressing CYP3A5 may have higher clearance and lower bioavailability of CYP3A substrates compared with individuals not expressing CYP3A5 (Kuehl et al., 2001). This has been demonstrated for both the immunosuppressive drug tacrolimus and for several HMG-CoA reductase inhibitors (statins) where patients expressing CYP3A5 need higher drug dosages to reach target serum concentration or to achieve sufficient lipid-lowering response, respectively (Hesselink et al., 2003; Zheng et al., 2003; Goto et al., 2004; Kivisto et al., 2004; Ferraresso et al., 2007).

CYP3A4 and CYP3A5 overlap in substrate specificity, but the relative importance of CYP3A4 and CYP3A5 in overall CYP3A-mediated metabolism differs between substrates (Lamba et al., 2002). The role of CYP3A5 in the metabolism of quetiapine is sparsely known. The objective of this *in vitro* study was therefore to investigate the relative importance of CYP3A4 and CYP3A5 in the metabolism of quetiapine, and to compare the metabolic pattern by the two enzymes. As the presence of cytochrome b<sub>5</sub> in the incubation assay has been shown to affect the metabolism of many drugs via CYP3A4 and CYP3A5 *in vitro* (Yamaori et al., 2003), the effect of cytochrome b<sub>5</sub> on quetiapine metabolism was also examined.

## Materials and methods

**Materials.** Quetiapine and N-desalkylquetiapine were purchased from Toronto Research Chemicals Inc. (North York, ON), quetiapine sulfoxide from Synthetica AS (Oslo, Norway) and promazine from Sigma-Aldrich (St. Louis, MO). Baculovirus insect cell microsomes expressing human CYP3A enzymes, cytochrome P450 reductase and cytochrome b<sub>5</sub> (Supersomes<sup>™</sup>) were purchased from BD Gentest (Woburn, MA), whereas similar CYP3A microsomes without coexpressed cytochrome b<sub>5</sub> (Xenosomes<sup>™</sup>) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden).

**Experimental conditions.** Preliminary metabolism studies of quetiapine using the metabolite formation method revealed a solubility problem of quetiapine, as the concentration of quetiapine in the buffer solutions was only about 30% of the nominal concentration. Organic solvents (dimethyl sulfoxide and methanol) were added to increase the solubility of quetiapine. The effect of 10% methanol on quetiapine metabolism was investigated, and an extensive inhibition (>40%) in the formation of the four metabolites by CYP3A4 and CYP3A5 was observed. Quetiapine metabolism was therefore instead examined by the substrate depletion approach, which requires low substrate concentrations (Jones and Houston, 2004). Quetiapine concentrations of 1  $\mu$ M in CYP3A4 microsomes and 5  $\mu$ M in CYP3A5 microsomes were well below K<sub>m</sub> for all metabolites (data not shown). These concentrations resulted in linear formation of the metabolites and provided quantifiable metabolite amounts.

Incubation assays contained either CYP3A4 or CYP3A5, with or without coexpressed cytochrome b<sub>5</sub>. Quetiapine (1  $\mu$ M for CYP3A4 and 5  $\mu$ M for CYP3A5) was incubated at 37°C in 118 mM Tris-H<sub>2</sub>SO<sub>4</sub> (pH 7.5), 0.5 mM MgSO<sub>4</sub> and 1.6 mM NADPH, incubation conditions optimized by Hermann et al. (2006). Microsomes were diluted in a solution (pH 7.4) consisting of 0.25 M sucrose, 10 mM HEPES and 2 mM EDTA. The reaction was initiated

by adding preheated microsomes, and final enzyme concentration was 4 nM in the total incubation volume of 220  $\mu$ l. Reactions were terminated after 0-60 min with 150  $\mu$ l ice-cold acetonitrile including internal standard (promazine). The concentration of internal standard was 4  $\mu$ M in each sample. After at least 30 min on ice, the samples were centrifuged at 4°C; 1600 g for 5 min (Universal 32R, Hettich zentrifugen, Tuttlingen, Germany), and 5  $\mu$ l of the supernatant was injected into the LC-MS/MS instrument. For each of the four microsomal preparations studied, three separate experiments were performed. All incubations in each experiment were performed in duplicate.

**Determination of quetiapine and metabolites.** Quetiapine and metabolites were analyzed on a 2795 liquid chromatograph and a Micromass Quattro Micro tandem mass spectrometer (both from Waters Corporation, Milford, MA). The analytes were separated on an ACE 3AQ C<sub>18</sub> analytical column (Advanced Chromatography Technologies, Aberdeen, Scotland) protected by an Atlantis dC<sub>18</sub> guard column (Waters). The samples were eluted at a flow rate of 0.25 ml/min by a gradient consisting of 25-80% acetonitrile in ammonium acetate (10 mM, pH 4.5). Total run time was 15 min. The retention times were 2.8 min for 7-hydroxyquetiapine, 3.1 min for quetiapine sulfoxide, 5.6 min for N-desalkylquetiapine, 5.7 min for O-desalkylquetiapine, 6.0 min for quetiapine and 6.9 min for promazine. Detection was performed in the multiple reaction monitoring mode at the transitions: 400→269 for 7-hydroxyquetiapine, 400→221 for quetiapine sulfoxide, 296→210 for N-desalkylquetiapine, 340→253 for O-desalkylquetiapine, 384→253 for quetiapine and 285→212 for promazine. Processing was performed with QuanLynx software version 4.0. Quetiapine, quetiapine sulfoxide and N-desalkylquetiapine were quantified using quadratic standard curves with a 1/Y weighting. For quetiapine, quetiapine sulfoxide and N-desalkylquetiapine, the validation data for intra- and interrun accuracy and precision were  $\leq$ 16% at the limit of quantification (LOQ, 0.1  $\mu$ M for quetiapine, and 0.01  $\mu$ M for quetiapine sulfoxide and N-

desalkylquetiapine), and  $\leq 9\%$  above LOQ. Reference substances of O-desalkylquetiapine and 7-hydroxyquetiapine were not available and these metabolites were therefore quantified using peak area ratio (metabolite/internal standard). Any deviation from linearity in MS-response will not be revealed using peak area ratio, implying more uncertainty in these results compared to the results based on standard curves.

**Data analysis.** Substrate depletion data were fitted to a monoexponential decay model (eq. 1), with a 1/Y weighting using GraphPad Prism version 4.03.

$$C_t = C_0 e^{-kt} \quad (1)$$

In each experiment the velocity constant  $k$  ( $\text{min}^{-1}$ ) for the substrate depletion was estimated and intrinsic clearance,  $CL_{\text{int}}$ , calculated (eq. 2).

$$CL_{\text{int}} = kV, \quad (2)$$

where  $V$  is the incubation volume.

The substrate depletion curves were log-transformed to visualize how well the monoexponential decay model described the substrate depletion data. The log-transformed substrate depletion curves were linear in the entire time-range for all microsomal preparations, except for CYP3A4 microsomes without cytochrome  $b_5$  after 40 min incubation (data not shown). Substrate depletion was therefore studied for 0-40 min in all four microsomal preparations. The metabolic pattern by CYP3A4 and CYP3A5 was compared by assessment of relative formation of the four metabolites of quetiapine after 40 min incubation in the different microsomal preparations.

Unpaired t-test was used to determine any statistical difference in  $CL_{\text{int}}$  of quetiapine by CYP3A4 in microsomes with or without coexpressed cytochrome  $b_5$ , and correspondingly for CYP3A5.  $P < 0.05$  was considered statistically significant.

## Results

**Determination of  $CL_{int}$ .** Substrate depletion studies with quetiapine by CYP3A4 and CYP3A5 showed monoexponential decay in microsomes with and without coexpressed cytochrome  $b_5$  ( $r^2 > 0.9$  for CYP3A4 and  $r^2 > 0.5$  for CYP3A5) (Fig. 2).

$CL_{int}$  for CYP3A4 and CYP3A5 with coexpressed cytochrome  $b_5$  was 3.2 and 1.0  $\mu\text{l}/\text{min}/\text{pmol}$  CYP, respectively (Table 1).  $CL_{int}$  of quetiapine by CYP3A5 was 32% (S.E.M. 7%) relative to CYP3A4. The corresponding  $CL_{int}$  values for CYP3A4 and CYP3A5 without coexpressed cytochrome  $b_5$  were 8.8 and 1.07  $\mu\text{l}/\text{min}/\text{pmol}$  CYP, respectively (Table 1), and  $CL_{int}$  for CYP3A5 was 12% (S.E.M. 1%) compared to CYP3A4.  $CL_{int}$  of quetiapine by CYP3A4 was significantly different ( $p < 0.0001$ ) in microsomes with coexpressed cytochrome  $b_5$  compared to microsomes without cytochrome  $b_5$  (Table 1), whereas for CYP3A5 no difference in  $CL_{int}$  was observed ( $p = 0.99$ ).

**Metabolic pattern.** Measurement of the four metabolites after 40 min incubation revealed differences in metabolic pattern by CYP3A4 and CYP3A5 (Fig. 3, Table 2). The main differences between CYP3A4 and CYP3A5 were observed for the formation of quetiapine sulfoxide and O-desalkylquetiapine. The formation of quetiapine sulfoxide was more than 8-fold higher by CYP3A4 compared to CYP3A5 both in microsomes with and without cytochrome  $b_5$  (Table 2). The opposite was observed for O-desalkylquetiapine, the formation was about 1.5-fold and 2.5-fold higher by CYP3A5 compared to CYP3A4 in microsomes with or without cytochrome  $b_5$ , respectively (Table 2). The formation of N-desalkylquetiapine and 7-hydroxyquetiapine was higher by CYP3A4 compared to CYP3A5 both in presence or absence of cytochrome  $b_5$  (Table 2).

## Discussion

This study demonstrates that quetiapine is metabolized by CYP3A5, but to a lesser degree than by CYP3A4.  $CL_{int}$  for quetiapine by CYP3A5 was less than 35% relative to CYP3A4 both in microsomes with and without coexpressed cytochrome  $b_5$ . Since CYP3A5 might constitute approximately 50% of total hepatic CYP3A protein in some individuals expressing CYP3A5 (Kuehl et al., 2001; Lin et al., 2002), theoretical clearance of quetiapine by CYP3A5 *in vivo* could accordingly be less than 35% relative to CYP3A4 in these individuals. Hence, this study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine *in vivo*. A previous *in vitro* study by Lin et al. (2004) also implied similar results, as they reported the metabolism of quetiapine by CYP3A5 to be approximately 10% compared to CYP3A4. However, this result is somewhat uncertain since Lin et al. compared quetiapine metabolism in CYP3A5 microsomes not expressing cytochrome  $b_5$  with CYP3A4 microsomes coexpressing cytochrome  $b_5$ .

$CL_{int}$  for quetiapine by the two enzymes varied dependent on the microsomal system studied. The metabolism of quetiapine was significantly different in CYP3A4 microsomes with coexpressed cytochrome  $b_5$  compared to in CYP3A4 microsomes without cytochrome  $b_5$ , whereas in CYP3A5 microsomes quetiapine was equally well metabolized independent of the presence of cytochrome  $b_5$ . Interestingly, the metabolism of quetiapine was more extensive in CYP3A4 microsomes without cytochrome  $b_5$ ;  $CL_{int}$  was almost 3-fold higher in these microsomes. However, the opposite has been reported in several studies, where a considerably increased metabolism in microsomal systems including cytochrome  $b_5$  has been demonstrated. For instance, Klees et al. (2005) reported a 5- to 7-fold increase in  $CL_{int}$  of alfentanil in CYP3A4 microsomes coexpressed with cytochrome  $b_5$ . Furthermore, the formation of the major metabolite of vincristine through CYP3A5 was stimulated (3-fold) by the presence of coexpressed cytochrome  $b_5$  (Dennison et al., 2006). The conflicting results

indicate a substrate dependent impact of cytochrome  $b_5$ , and this should be further evaluated for more substrates. Additionally, it would also be relevant to study if possible differences in composition of microsomal preparations, such as expression of cytochrome P450 reductase, may contribute to the conflicting data on the metabolic impact of cytochrome  $b_5$  *in vitro*.

The metabolic pattern was different for metabolism of quetiapine by CYP3A5 compared to CYP3A4. The most important difference was observed for O-desalkylquetiapine where the formation of O-desalkylquetiapine was higher by CYP3A5 compared to CYP3A4. Concurrently,  $CL_{int}$  of quetiapine was lower by CYP3A5 than by CYP3A4. These results indicate that O-desalkylquetiapine constituted a higher proportion of the formed metabolites by CYP3A5 compared to CYP3A4. Several studies of other substrates have also reported differences in metabolic pattern by the two enzymes. For instance, Huang et al. (2004) found a higher formation ratio of major metabolite to minor metabolite of midazolam by CYP3A5 than by CYP3A4. A difference in the metabolic pattern of quetiapine by CYP3A4 and CYP3A5 may theoretically affect the clinical outcome associated to CYP3A5 genotype, despite a limited impact of CYP3A5 on overall metabolic clearance of quetiapine. The results of the present study indicate a possible higher serum concentration of O-desalkylquetiapine in patients expressing CYP3A5. However, the pharmacological and toxicological activity of O-desalkylquetiapine is not known, nor is the serum concentration range of this metabolite following therapeutic use of quetiapine.

The substrate depletion method was applied in this study because of solubility problems of quetiapine with the traditional metabolite formation method. Substrate depletion has some disadvantages compared to typical kinetic estimations based on metabolite formation with increasing substrate concentrations. At least 20% of the substrate should be metabolized within the incubation period to distinguish substrate depletion from baseline variability (Jones and Houston, 2004). Consequently, longer incubation times and higher enzyme

concentrations are generally required (Jones and Houston, 2004). The depletion of quetiapine was less than 20% within the incubation period of 40 minutes in the CYP3A5 microsomes. However, longer incubation times (60 min) resulted in deviation from linearity of the log-transformed substrate depletion curves for CYP3A4 without cytochrome b<sub>5</sub>. Additionally, the enzyme concentration was kept at a low level to reduce the risk of non-specific binding.

The interindividual variability in serum concentration of quetiapine is substantial (Gerlach et al., 2007), but the results of the current study indicate that other factors besides genetic polymorphism in *CYP3A5* are determinant for this variability. However, discrepancies between *in vitro* and *in vivo* data are well-known for many substrates and could be caused by experimental conditions, interindividual variability, extrahepatic metabolism or nonlinear kinetics (Tucker et al., 2001; Donato and Castell, 2003). For instance, polymorphism in *CYP3A5* is shown to influence the systemic exposure and lipid-lowering response to simvastatin (Kivisto et al., 2004; Kim et al., 2007), although CYP3A4 possessed a 3- to 9-fold higher affinity than CYP3A5 for the formation of three major metabolites of simvastatin *in vitro* (Prueksaritanont et al., 1997). Consequently, clinical studies are necessary to elucidate whether genetic polymorphism in *CYP3A5* is a factor involved in the interindividual variability in serum concentration of quetiapine.

In conclusion, this *in vitro* study indicates that CYP3A5 is of minor importance for the overall metabolism of quetiapine, independent of the presence of cytochrome b<sub>5</sub> in the microsomal preparation. However, discrepancies between *in vitro* and *in vivo* data are common, and clinical studies are therefore necessary to exclude a possible impact of CYP3A5 on the metabolism of quetiapine *in vivo*. The metabolic pattern of quetiapine was different for metabolism by CYP3A5 compared to CYP3A4. This could possibly result in different pharmacological and/or toxicological effects of quetiapine in patients expressing CYP3A5.

## References

- Burk O and Wojnowski L (2004) Cytochrome P450 3A and their regulation. *Naunyn-Schmiedebergs Arch Pharmacol* **369**:105-124.
- Dennison JB, Kulanthaivel P, Barbuch RJ, Renbarger JL, Ehlhardt WJ and Hall SD (2006) Selective metabolism of vincristine in vitro by CYP3A5. *Drug Metab Dispos* **34**:1317-1327.
- DeVane CL and Nemeroff CB (2001) Clinical pharmacokinetics of quetiapine: an atypical antipsychotic. *Clin Pharmacokinet* **40**:509-522.
- Donato MT and Castell JV (2003) Strategies and molecular probes to investigate the role of cytochrome P450 in drug metabolism: focus on in vitro studies. *Clin Pharmacokinet* **42**:153-178.
- Ferraresso M, Tirelli A, Ghio L, Grillo P, Martina V, Torresani E and Edefonti A (2007) Influence of the Cyp3a5 genotype on tacrolimus pharmacokinetics and pharmacodynamics in young kidney transplant recipients. *Pediatr Transplant* **11**:296-300.
- Gefvert O, Bergstrom M, Langstrom B, Lundberg T, Lindstrom L and Yates R (1998) Time course of central nervous dopamine-D-2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel (R)) in patients with schizophrenia. *Psychopharmacology* **135**:119-126.
- Gerlach M, Hunnerkopf R, Rothenhofer S, Libal G, Burger R, Clement HW, Fegert JM, Wewetzer C and Mehler-Wex C (2007) Therapeutic drug monitoring of quetiapine in adolescents with psychotic disorders. *Pharmacopsychiatry* **40**:72-76.
- Goldstein JM, Christoph G, Grimm S, Liu JW, Widzowski D and Brecher M (2007) Unique mechanism of action for the antidepressant properties of the atypical antipsychotic

quetiapine, in: *The Society of Biological Psychiatry's 62nd Annual Convention & Scientific Program*, San Diego, CA, USA.

Goto M, Masuda S, Kiuchi T, Ogura Y, Oike F, Okuda M, Tanaka K and Inui K (2004)

CYP3A5\*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. *Pharmacogenetics* **14**:471-478.

Grimm SW, Richtand NM, Winter HR, Stams KR and Reece SB (2006) Effects of cytochrome P450 3A modulators ketoconazole and carbamazepine on quetiapine pharmacokinetics. *Br J Clin Pharmacol* **61**:58-69.

Grimm SW, Stams KR and Bui K (1997) In vitro prediction of potential metabolic drug interactions for Seroquel. *Schizophr Res* **24**:198.

Hermann M, Kase ET, Molden E and Christensen H (2006) Evaluation of microsomal incubation conditions on CYP3A4-mediated metabolism of cyclosporine A by a statistical experimental design. *Curr Drug Metab* **7**:265-271.

Hesselink DA, van Schaik RHN, van der Heiden IP, van der Werf M, Gregoor P, Lindemans J, Weimar W and van Gelder T (2003) Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* **74**:245-254.

Huang W, Lin YS, McConn II DJ, Calamia JC, Totah RA, Isoherranen N, Glodowski M and Thummel KE (2004) Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. *Drug Metab Dispos* **32**:1434-1445.

Jensen NH, Rodriguiz RM, Caron MG, Wetsel WC, Rothman RB and Roth BL (2008) N-Desalkylquetiapine, a potent norepinephrine reuptake inhibitor and partial 5-HT<sub>1A</sub> agonist, as a putative mediator of quetiapine's antidepressant activity. *Neuropsychopharmacology* **33**:2303-2312.

- Jones HM and Houston JB (2004) Substrate depletion approach for determining in vitro metabolic clearance: Time dependencies in hepatocyte and microsomal incubations. *Drug Metab Dispos* **32**:973-982.
- Kim KA, Park PW, Lee OJ, Kang DK and Park JY (2007) Effect of polymorphic CYP3A5 genotype on the single-dose simvastatin pharmacokinetics in healthy subjects. *J Clin Pharmacol* **47**:87-93.
- Kivisto KT, Niemi M, Schaeffeler E, Pitkala K, Tilvis RT, Fromm MF, Schwab M, Eichelbaum M and Strandberg T (2004) Lipid-lowering response to statins is affected by CYP3A5 polymorphism. *Pharmacogenetics* **14**:523-525.
- Klees TM, Sheffels P, Dale O and Kharasch ED (2005) Metabolism of alfentanil by cytochrome p4503a (cyp3a) enzymes. *Drug Metab Dispos* **33**:303-311.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS and Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27**:383-391.
- Lamba JK, Lin YS, Schuetz EG and Thummel KE (2002) Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* **54**:1271-1294.
- Lin S-N, Chang Y, Moody DE and Foltz RL (2004) A liquid chromatographic-electrospray-tandem mass spectrometric method for quantitation of quetiapine in human plasma and liver microsomes: application to study in vitro metabolism. *J Anal Toxicol* **28**:443-448.
- Lin YS, Dowling ALS, Quigley SD, Farin FM, Zhang J, Lamba J, Schuetz EG and Thummel KE (2002) Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. *Mol Pharmacol* **62**:162-172.

- McIntyre RS, Soczynska JK, Woldeyohannes HO, Alsuwaidan M and Konarski JZ (2007) A preclinical and clinical rationale for quetiapine in mood syndromes. *Expert Opin Pharmacother* **8**:1211-1219.
- Prueksaritanont T, Gorham LM, Ma B, Liu LD, Yu X, Zhao JGJ, Slaughter DE, Arison BH and Vyas KP (1997) In vitro metabolism of simvastatin in humans [SBT]identification of metabolizing enzymes and effect of the drug on hepatic P450S. *Drug Metab Dispos* **25**:1191-1199.
- Tucker GT, Houston JB and Huang SM (2001) Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential-toward a consensus. *Clin Pharmacol Ther* **70**:103-114.
- Westlind-Johnsson A, Malmebo S, Johansson A, Otter C, Andersson TB, Johansson I, Edwards RJ, Boobis AR and Ingelman-Sundberg M (2003) Comparative analysis of CYP3A expression in human liver suggests only a minor role for CYP3A5 in drug metabolism. *Drug Metab Dispos* **31**:755-761.
- Yamaori S, Yamazaki H, Suzuki A, Yamada A, Tani H, Kamidate T, Fujita K and Kamataki T (2003) Effects of cytochrome b(5) on drug oxidation activities of human cytochrome P450 (CYP) 3As: similarity of CYP3A5 with CYP3A4 but not CYP3A7. *Biochem Pharmacol* **66**:2333-2340.
- Zheng HX, Webber S, Zeevi A, Schuetz E, Zhang J, Bowman P, Boyle G, Law Y, Miller S, Lamba J and Burckart GJ (2003) Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and MDR1 gene polymorphisms. *Am J Transplant* **3**:477-483.

### Figure legends

FIG. 1. Metabolism of quetiapine *in vitro*

FIG. 2. Substrate depletion curves for quetiapine in CYP3A4 and CYP3A5 microsomes with or without coexpressed cytochrome b<sub>5</sub> (cyt b<sub>5</sub>). The initial concentration of quetiapine was 1  $\mu$ M in CYP3A4 microsomes and 5  $\mu$ M in CYP3A5 microsomes. Data are presented as mean  $\pm$  S.E.M. (n=3)

FIG. 3. Formation of quetiapine sulfoxide, N-desalkylquetiapine, O-desalkylquetiapine and 7-hydroxyquetiapine in CYP3A4 and CYP3A5 microsomes with or without coexpressed cytochrome b<sub>5</sub> (cyt b<sub>5</sub>). Data for quetiapine sulfoxide and N-desalkylquetiapine are metabolite concentration ( $\mu$ M), whereas data for O-desalkylquetiapine and 7-hydroxyquetiapine are arbitrary (peak area ratio). The metabolite formation data is divided with quetiapine concentration (1  $\mu$ M for CYP3A4 and 5  $\mu$ M for CYP3A5); correspondingly the data for CYP3A4 and CYP3A5 are comparable. Data are presented as mean metabolite formation  $\pm$  S.E.M. (n=3) in each of the four microsomal preparations.

## Tables

TABLE 1.

*CL<sub>int</sub> for substrate depletion of quetiapine in CYP3A4 and CYP3A5 microsomes with or without coexpressed cytochrome b<sub>5</sub>*

Data are presented as mean ± S.E.M. (n=3).

Microsomal preparation	CL <sub>int</sub> (μl/min/pmol CYP)
CYP3A4 + cyt b <sub>5</sub>	3.2 ± 0.1
CYP3A4	8.8 ± 0.4
CYP3A5 + cyt b <sub>5</sub>	1.0 ± 0.2
CYP3A5	1.07 ± 0.02

\* Significantly different CL<sub>int</sub> (p<0.0001)

TABLE 2.

*Metabolite formation in CYP3A4 and CYP3A5 microsomes with or without coexpressed cytochrome b<sub>5</sub> after 40 min incubation of quetiapine*

The metabolite formation data are divided with quetiapine concentration (1  $\mu$ M for CYP3A4 and 5  $\mu$ M for CYP3A5); correspondingly the data for CYP3A4 and CYP3A5 are comparable. Data for quetiapine sulfoxide and N-desalkylquetiapine are metabolite concentration ( $\mu$ M), whereas data for O-desalkylquetiapine and 7-hydroxyquetiapine are arbitrary (peak area ratio). Data are presented as mean  $\pm$  S.E.M. (n=3).

Metabolite	CYP3A4 + cyt b <sub>5</sub>	CYP3A4	CYP3A5 + cyt b <sub>5</sub>	CYP3A5
Quetiapine sulfoxide	0.233 $\pm$ 0.018	0.304 $\pm$ 0.017	0.028 $\pm$ 0.001	0.0376 $\pm$ 0.0005
N-desalkylquetiapine	0.072 $\pm$ 0.004	0.157 $\pm$ 0.018	0.0158 $\pm$ 0.0002	0.025 $\pm$ 0.003
O-desalkylquetiapine	0.049 $\pm$ 0.014	0.057 $\pm$ 0.013	0.068 $\pm$ 0.015	0.142 $\pm$ 0.043
7-hydroxyquetiapine	0.008 $\pm$ 0.004	0.018 $\pm$ 0.006	0.003 $\pm$ 0.001	0.007 $\pm$ 0.003

Fig. 1

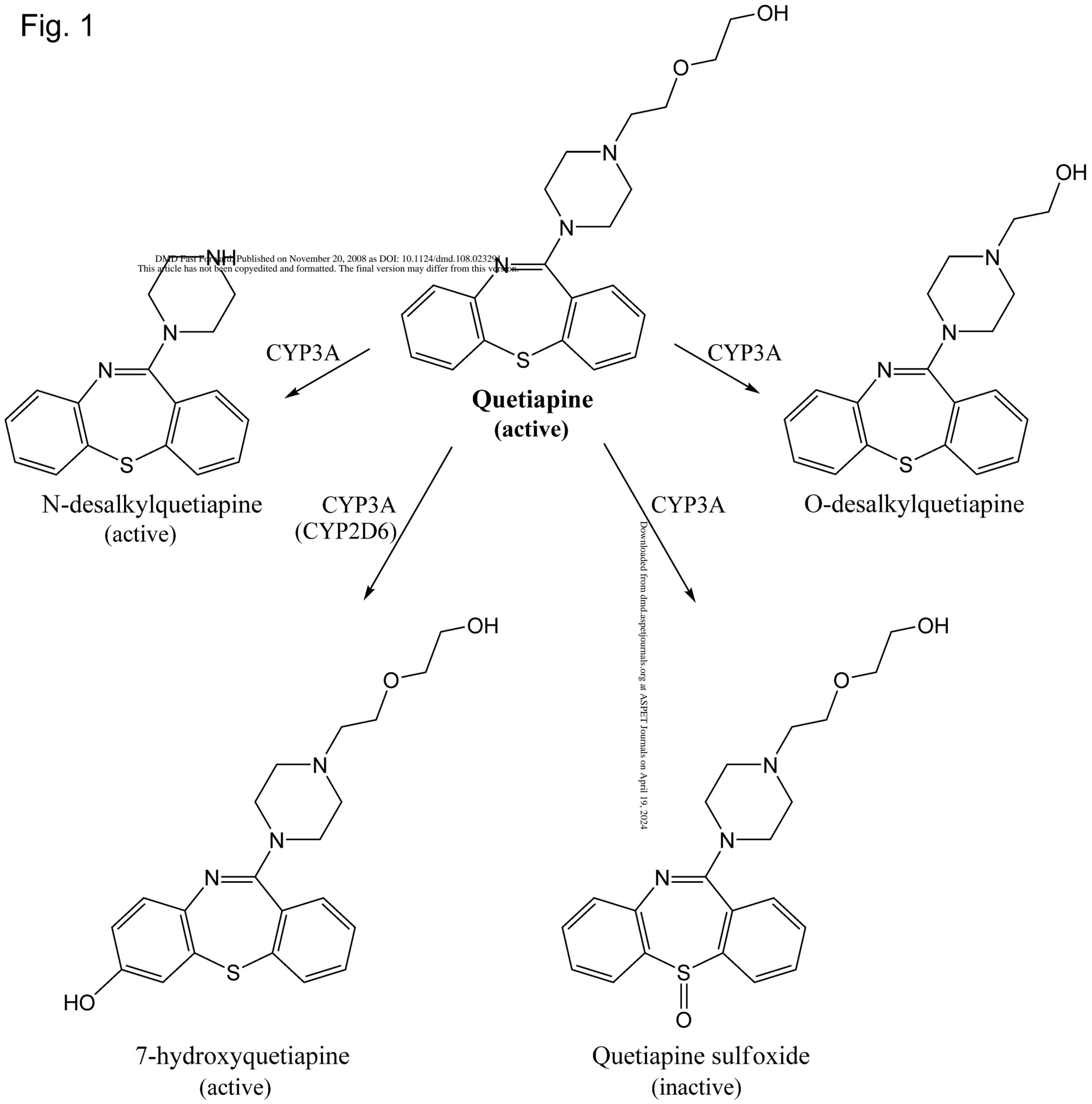
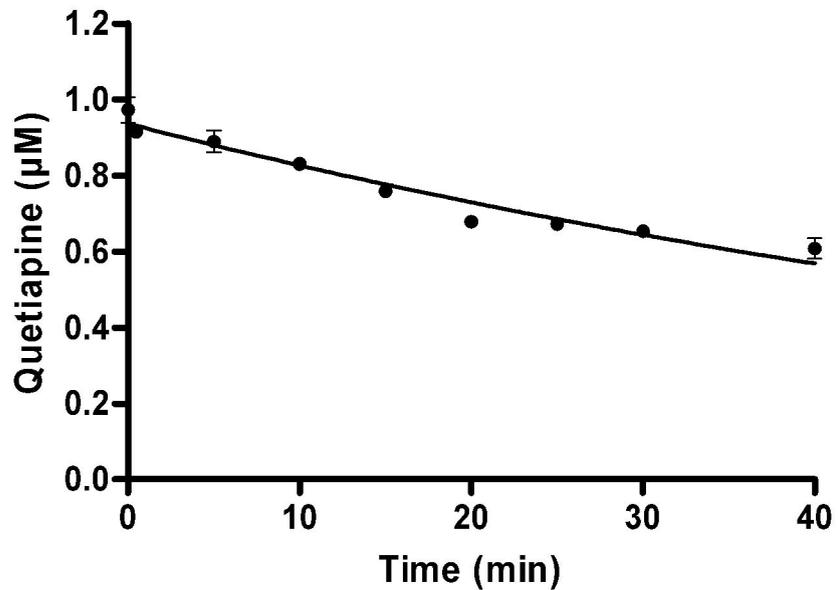
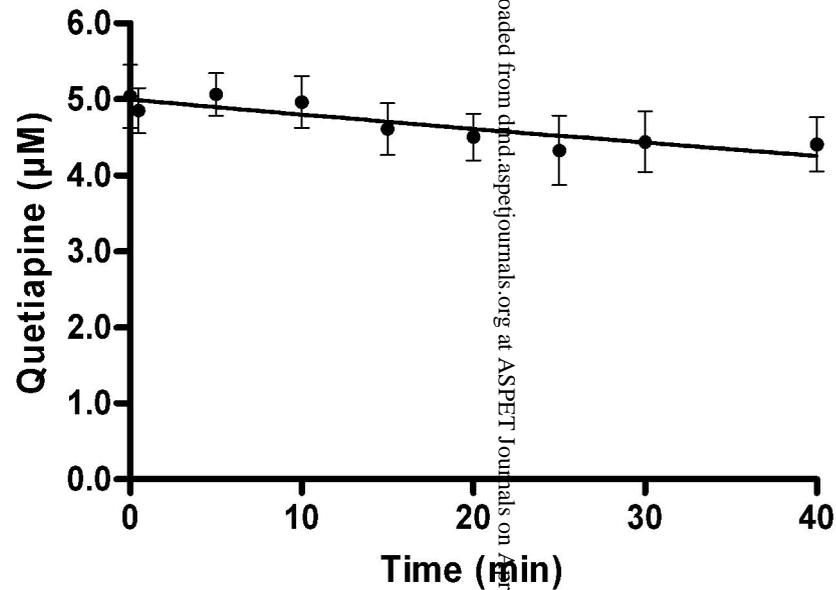


Fig. 2

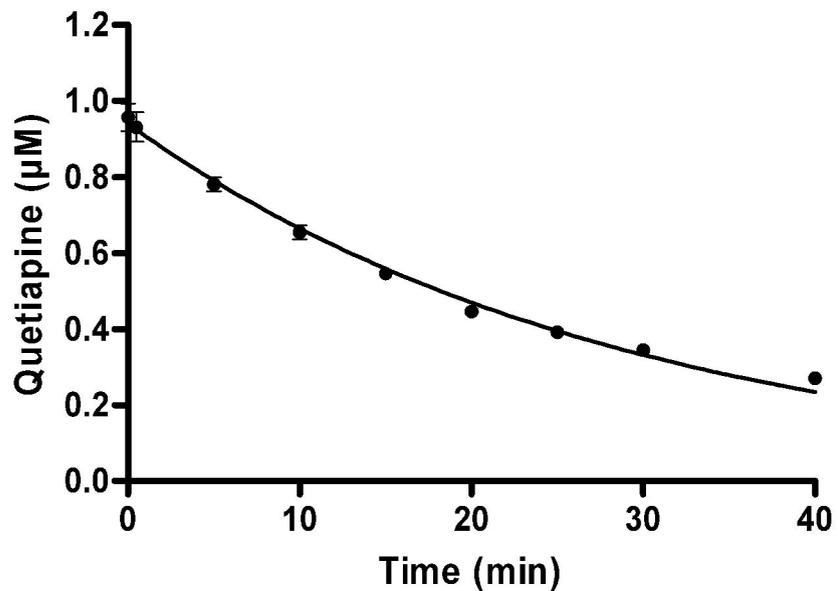
**CYP3A4 + cyt b<sub>5</sub>**



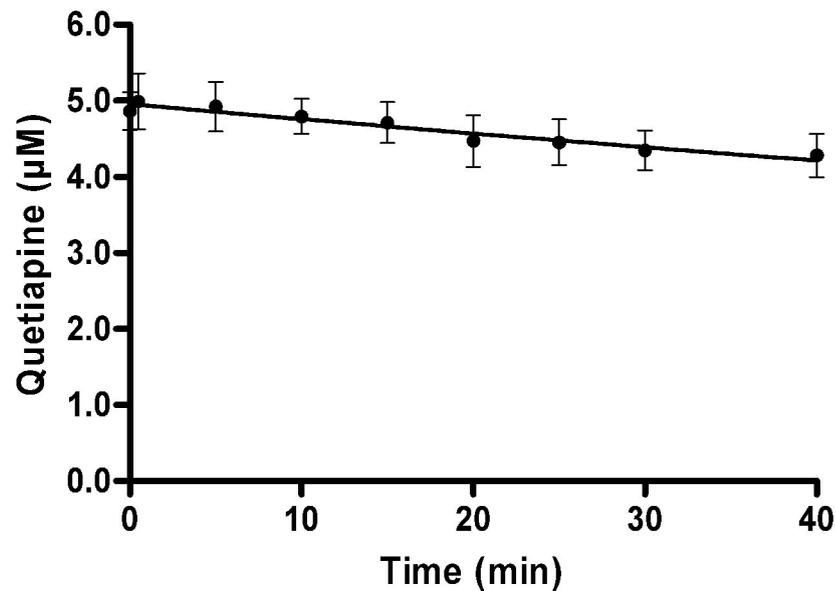
**CYP3A5 + cyt b<sub>5</sub>**



**CYP3A4**



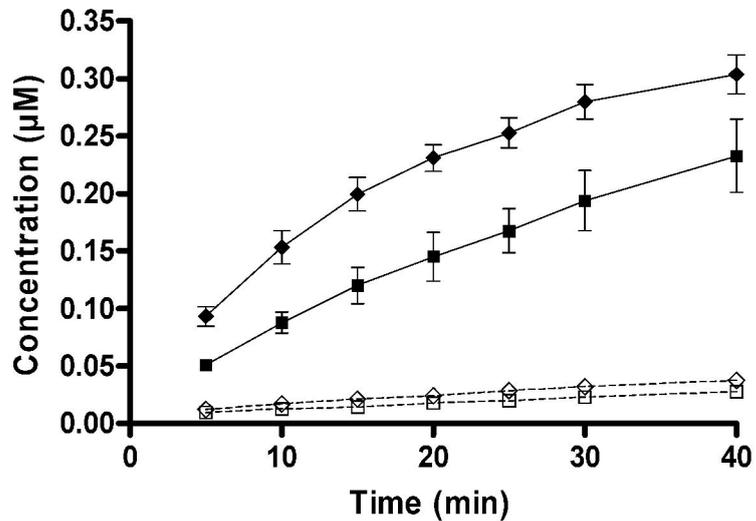
**CYP3A5**



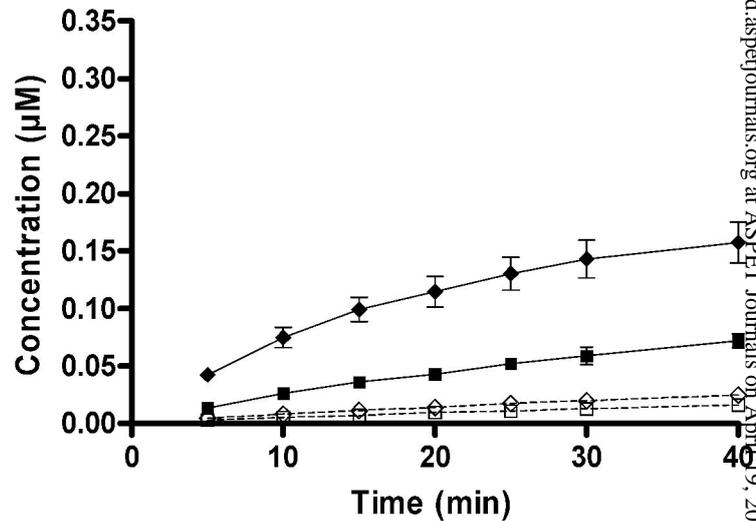
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Fig. 3

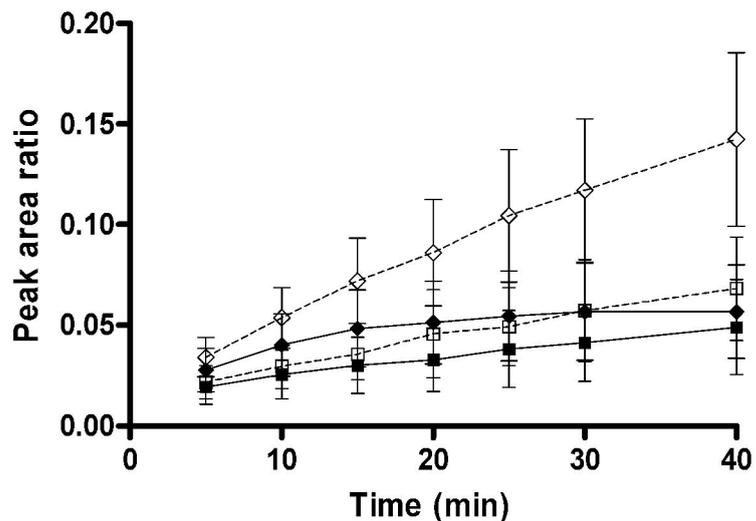
### Quetiapine sulfoxide



### N-desalkylquetiapine



### O-desalkylquetiapine



### 7-hydroxyquetiapine

