The N-Demethylation of the Doxepin Isomers Is Mainly Catalyzed by the Polymorphic CYP2C19

Sebastian Härtter,[1,4] Gunnel Tybring,[2] Thomas Friedberg,[3] Harald Weigmann,[1] and Christoph Hiemke[1]

Received November 28, 2001; accepted April 1, 2002

Purpose. This study was conducted to identify the cytochrome P450s (CYPs) responsible for the metabolism of the cis- and trans-isomers of the tricyclic antidepressant doxepin to its pharmacologically active N-desmethylmetabolite by *in vitro* techniques.

Methods. The doxepin N-demethylation was studied by means of pooled human liver microsomes and chemical inhibitors, recombinant human (rh)-CYPs, and geno- and phenotyped human liver microsomes.

Results. The N-demethylation of both isomers was inhibited most prominently by tranylcypromine (CYP2C19) to more than 50%. Furafylline (CYP1A2) and sulfaphenazole (CYP2C9) inhibited the N-demethylation to a lesser extent while quinidine (CYP2D6) or troleandomycine (CYP3A4) had no effect. Rh-CYP2C19, -CYP1A2, and -CYP2C9 were able to N-demethylate cis- and trans-doxepin. Only traces of trans-desmethyldoxepin were detectable when CYP3A4 was used. The maximum velocity in the cis- and trans-doxepin N-demethylation was significantly ($P < 0.05$) lower in microsomes with low CYP2C19 activity (345 ± 44 and 508 ± 75 pmol/min/mg protein, respectively) compared to those with high CYP2C19 activity (779 ± 132 and 1189 ± 134 pmol/min/mg).

Conclusion. The present study demonstrates a significant contribution of the polymorphic CYP2C19 to the N-demethylation of doxepin. CYP2C9 and CYP1A2 play a minor role and CYP3A4 does not contribute substantially.

KEY WORDS: doxepin; cis-/trans-isomers; *in vitro*; metabolism; tricyclic antidepressants.

INTRODUCTION

Doxepin, a tricyclic antidepressant (TCA), is marketed as a 15:85% mixture of its Z-(cis) and E-(trans-) isomer although the former is suggested to be the pharmacologically more active isomer. Beside serotonin and noradrenalin reuptake, inhibition doxepin is one of the most potent antagonists at the H1-histamine receptor leading to a high sedative potential (1). Doxepin undergoes extensive metabolism predominantly N-demethylation and aromatic ring hydroxylation (2) as shown in Fig. 1. The predominant metabolite found in serum of patients taking doxepin is N-desmethyldoxepin which normally is equal to or even exceeds the concentration of the parent drug. However, in contrast to the parent drug the cis-isomer makes up about 50% of the total N-desmethyldoxepin (3). Interconversion of the trans- to the cis-isomer was proposed (4). The accumulation of the cis-isomer is, however, more likely caused by a much faster metabolism of trans-N-desmethyldoxepin (5).

So far, only little is known about the enzymes catalyzing the main metabolic step, the N-demethylation of doxepin (6). This is surprising as doxepin is combined with other drugs some of them are known inhibitors of distinct CYPs like fluvoxamine (7).

To predict drug-drug interactions in patients and avoid problems in the therapy it is a prerequisite to know the enzymes contributing to the metabolism of the affected drug. This study was therefore conducted to identify the CYP(s) responsible for the formation of the main metabolites in serum, cis-, and trans-N-desmethyldoxepin. Because the polymorphic CYP2C19 is one of the major CYPs catalyzing the N-demethylation of other TCAs like imipramine (8) this study was especially focused on the role of CYP2C19 in the N-demethylation of the doxepin isomers.

MATERIALS AND METHODS

Chemicals

Cis/trans-doxepin-HCl was provided by Boehringer-Mannheim (Mannheim, Germany); cis-desmethyldoxepin and trans-desmethyldoxepin were gifts from Pfizer Inc. (Groton, USA). Diazepam and its metabolites N-desmethyldiazepam, temazepam, and oxazepam were provided by Roche (Basel, Switzerland). Tranylcypromine and furafylline were obtained from RBI (Natick, USA), phenethyl isothiocyanate and troleandomycin from Sigma (St. Louis, Missouri), and NADPH was obtained from Boehringer-Mannheim. All other chemicals like dimethylsulfoxide and HPLC solvents were at least of analytical grade and obtained from Merck (Darmstadt, Germany).

Microsomes

Microsomes from 20 different liver samples were CYP2C19 genotyped and the CYP2C19 activity was assessed by means of the 5-hydroxylation of R-omeprazole (single concentration of 5.4 μM) based on the *in vivo* phenotyping method as described earlier (9). Out of this pool, liver microsomes from six different donors were chosen with either the highest (n = 3) or the lowest (n = 3) omeprazole 5-hydroxylation activity. All were genotypically CYP2C19 EM and only one was heterozygous *1/*2.

A pool (HLM) of liver microsomes from six different human livers (Cat. No. H161, Lot 4), was purchased from Gentest Corporation (Woburn, USA) and used for enzyme kinetics and with the chemical inhibitors.

Recombinant human (rH)-CYP1A2, -CYP2C9, -CYP2C19, -CYP2D6, -CYP2E1, and -CYP3A4, co-expressed with human reductase, were prepared at the Biomedical Research Center, Ninewells Hospital (Dundee, Scotland, United Kingdom). The catalytic activity of the rH-CYPs is reported elsewhere (10).

[1] Department of Psychiatry, University of Mainz, Untere Zahlbacherstraße 8, 55131-Mainz, Germany.
[2] Department of Medical Laboratory Sciences and Technology, Division of Clinical Pharmacology, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden.
[3] Biomedical Research Center, Ninewells Hospital and Medical School, Dundee, Scotland.
[4] To whom correspondence should be addressed: (e-mail: sepp@psychiatrie.klinik.uni-mainz.de)

0724-8741/02/0700-1034/0 © 2002 Plenum Publishing Corporation

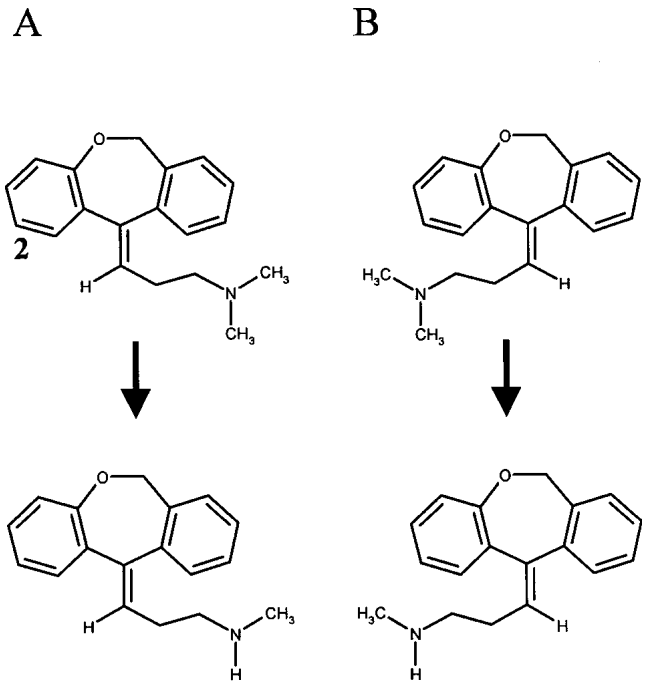

Fig. 1. Metabolic fate of trans- (A, left side) and cis-doxepin (B) to the main metabolites cis- and trans N-desmethyldoxepin. Trans-doxepin is also hydroxylated at position 2. No hydroxymetabolite of the cis-isomer has been described, so far.

Incubations Using Human Liver Microsomes

Incubations were performed at 37°C using 1 mg/ml protein following standard procedures. The reaction was quenched after 60 min by addition of 0.5 ml of the HPLC eluent (hexane, methanol, nonylamine 95, 5, 0.3, v/v/v) and rapid cooling on ice. After addition of the internal standard N-desmethylmaprotiline (5 μg/ml) samples were centrifuged and the organic supernatant was directly used for chromatographic analysis. The formation of cis- N-desmethyldoxepin was found to be linear for up to 60 min and 1 mg protein while the formation of trans-N-desmethyldoxepin was linear for up to 2 mg protein. Cis/trans-doxepin was used in a concentration range between 1 and 250 μM which is equivalent to a concentration range between 0.15–37.5 μM of the cis-isomer and between 0.85–212.5 μM of the trans-isomer. The formation of the metabolites cis- and trans-N-desmethyldoxepin was assayed by means of normal phase HPLC with UV-detection at 254 nm according to the method of Yan *et al.* (11). The limit of quantification was about 0.1 μM, which was sufficient for our purpose. Each incubation was accompanied by 5 calibration samples (cis- and trans-N-desmethyldoxepin) in a concentration range between 0.188–3.77 μM (50–3000 ng/ml) prepared and processed exactly the same as the incubated samples. The mean coefficient of variation of the calibration samples (n = 21 different days) was 12 ± 6% for the cis- and 12 ± 9% for the trans-isomer.

Liver microsomes characterized for their CYP2C19 activity were incubated with cis/trans-doxepin in a concentration range between 10–500 μM and the resulting kinetic parameters substrate concentration of half maximal velocity (Km) and maximal velocity (Vmax) were correlated with the formation rate of R-5-hydroxyomeprazole and omeprazole sulfone after incubation of 5 μM omeprazole.

The velocities of the enzyme reactions (v) were determined from the time-dependent formation of the products cis- and trans-N-desmethyldoxepin. Km and Vmax were estimated by fitting the untransformed data to the equations:

$$v = Vmax1 * S/(Km1 + S) + Vmax2 * S/(Km2 + S)$$

or

$$v = Vmax * S/(Km + S)$$

by means of nonlinear regression analysis using the GraFit program (version 4.03, Erithacus Software Ltd., Staines, United Kingdom).

All incubations were carried out in duplicate with a maximum intraassay deviation in the formation of desmethyldoxepin of 10% at the lowest substrate concentration (1 μM cis/trans doxepin).

Inhibition Experiments

The chemical inhibitor quinidine (CYP2D6 specific) was used in a concentration of 1 μM, furafylline (CYP1A2 specific) and sulfaphenazole (CYP2C9 specific) were used in concentrations of 10 μM, dimethylsulfoxide, phenethyl isothiocyanate (both CYP2E1, not specific) and tranylcypromine (CYP2C19, not specific) in a concentration of 50 μM and troleandomycin (CYP3A4 specific) in a concentration of 100 μM. When mechanism-based inhibitors (phenethyl isothiocyanate, furafylline, or troleandomycin) were applied, a 10 min preincubation was carried out and the reaction started by addition of the substrate.

Inhibitions were performed at two concentrations of cis/trans-doxepin (5 and 20 μM). These concentrations corresponded to the assumed maximal concentration of doxepin in the portal vein after an oral dose of either 50 or 200 mg and no intestinal metabolism according to the findings with midazolam where the maximal concentration in portal vein was about 0.1 μM after administration of 2 mg orally (12).

Because the specificity and inhibitory effect of the chemical inhibitor tranylcypromine was somewhat unclear the inhibition by tranylcypromine was checked by diazepam N-demethylation (13). While tranylcypromine (50 μM) reduced the CYP2C19 mediated formation of nordiazepam by 70%, its effect on the CYP3A4 mediated formation of temazepam was negligible.

Incubations Using Recombinant CYPs

Because linearity in the formation of N-desmethyldoxepin was not given for some of the recombinant CYPs (2C19 and 2C9) using 60 min incubation time incubation experiments were performed at two substrate concentrations (5 and 20 μM) using 20 min incubation time where linearity was given for all recombinant CYPs. Based on the amount of total P450 determined in the pooled microsomes used for the inhibition experiments (308 pmol/mg protein = 154 pmol/assay) the respective amount of the recombinant-CYPs were used according to their abundance in human livers (14). The amount of P450/assay was 20 pmol for CYP1A2, 17 pmol for CYP2C9, 8 pmol for CYP2C19, 3 pmol for CYP2D6, 11 pmol for CYP2E1, and 45 pmol for CYP3A4.

RESULTS

With regard to the formation of cis- and trans-N-desmethyldoxepin, a non-linear kinetic became visible in the resulting Eadie-Hofstee plots pointing to an at least biphasic enzyme system. Resulting Km and Vmax for the high affinity site were 0.4 μM and 12.5 pmol/min ∗ mg protein for the formation of cis-N-desmethyldoxepin and 15.7 μM and 60 pmol/min/mg protein for the formation of trans-N-desmethyldoxepin. Km and Vmax for the low affinity site were 44 μM and 235 pmol/min ∗ mg and 164 μM and 653 pmol/min ∗ mg for cis- and trans-N-desmethyldoxepin, respectively.

The most prominent inhibitory effect was found with tranylcypromine and phenethyl isothiocyanate (Fig. 2). The ratio of the two isomers did not show any differences to that seen without inhibitor. The N-demethylation of doxepin was also inhibited by sulfaphenazole and furafylline while quinidine, dimethylsulfoxide, and troleandomycin had no effect on the formation of N-desmethyldoxepin (Fig. 2).

In agreement with the inhibition experiments, rH-CYP2C19 and to a lesser extent rH-CYP1A2 and rH-CYP2C9 were able to N-demethylate DOX as shown in Fig. 3. When rH-CYP3A4 was assayed, only low amounts of trans-DDOX were measurable at the high substrate concentration. No N-desmethyldoxepin was formed when rh-CYP2D6 and rh- CYP2E1 were used. Percentage cis-N-desmethyldoxepin of total N-desmethyldoxepin was 19% when rH-CYP1A2, 21% when rH-CYP2C9 and 32% when rH-CYP2C19 was applied. Regarding the formation of either cis- or trans-N-desmethyldoxepin microsomes with low CYP2C19 activity could be clearly distinguished from the group with high CYP2C19 activity. (Fig. 4). The Vmax of cis- and trans-N-desmethyldoxepin was significantly lower in the CYP2C19 low activity group ($P < 0.05$ and $P < 0.01$, respectively).

The Vmax of both, the formation of cis-N-desmethyldoxepin and trans-N-desmethyldoxepin was significantly correlated with the formation rate of R-5-hydroxyomeprazole. ($R = 0.839$ and $R = 0.844$; $P < 0.05$) but not with the formation of omeprazole sulfone ($R = -0.037$ and 0.201, respectively). The Kms were slightly higher in the low activity group (109 ± 50 μM for cis-DOX and 511 ± 76 μM for trans-DOX) compared to the high activity group (79 ± 15 for cis-DOX and 415 ± 74 μM for trans-DOX).

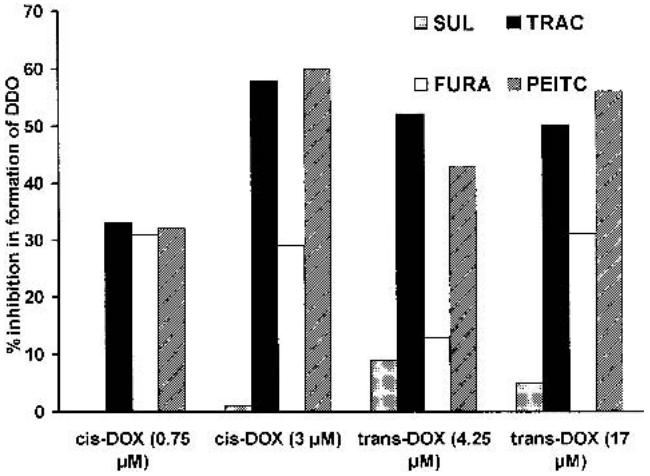

Fig. 2. Percent inhibition (compared to incubations without inhibitor) of the formation of either cis- or trans-N-desmethyldoxepin (DDOX) after incubation of 5 or 20 μM cis/trans-doxepin (DOX) -corresponding to 0.75 and 3 μM cis-doxepin and 4.25 and 17 μM trans-doxepin- in the presence of the probe inhibitors: 10 μM furafylline (FURA, white bars), 10 μM sulfaphenazole (SUL, grey bars), 50 μM tranylcypromine (TRAC, black bars), or 50 μM phenethyl isothiocyanate (PEITC, shaded bars). No inhibition was found with 50 μM dimethylsulfoxide, 1 μM quinidine, or 100 μM troleandomycin. Given is the mean of duplicate incubations.

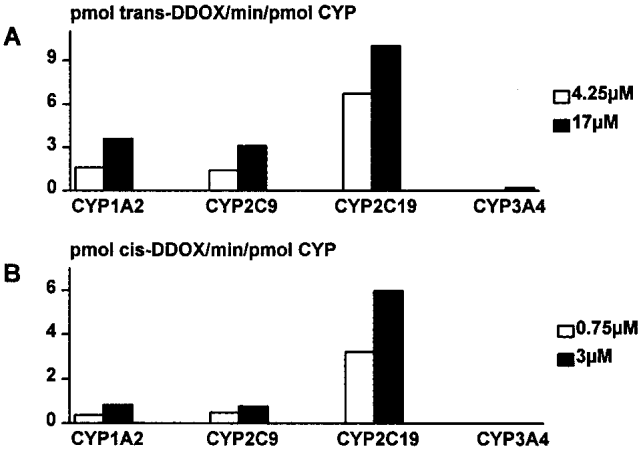

Fig. 3. Formation of (A) trans-N-desmethyldoxepin (trans-DDOX) and (B) cis-N-desmethyldoxepin (cis-DDOX) after incubation of 5 (white bars) or 20 μM (black bars) cis/trans-doxepin (corresponding to 0.75 and 3 μM cis-doxepin and 4.25 and 17 μM trans-doxepin) with different recombinant-CYPs for 20 min. No DDOX was found after incubation with recombinant human CYP2D6 and CYP2E1. Given is the means of duplicate incubations.

DISCUSSION

This study provides evidence that CYP2C19 contributes substantially to the N-demethylation of cis- and trans-doxepin. CYP1A2 and possibly CYP2C9 seem also to cata-

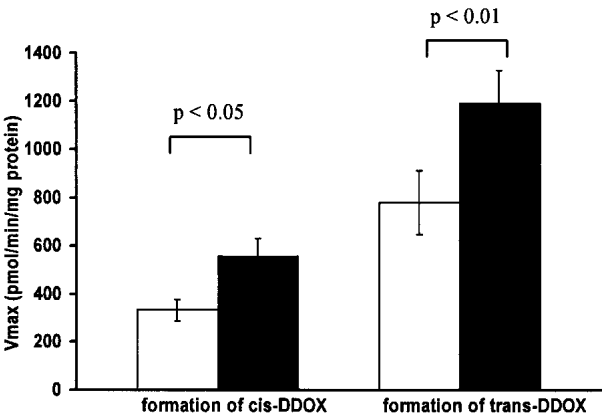

Fig. 4. Mean (± SD) maximum velocity (Vmax) of the formation of either cis-desmethyldoxepin (cis-DDOX) or trans-desmethyldoxepin (trans-DDOX) after incubation of cis-/trans-doxepin in a concentration between 10 and 500 μM with CYP2C19 phenotyped human liver microsomes. High CYP2C19 (black bars) refers to an exceptional high rate of the CYP2C19 catalyzed 5-hydroxylation of R-omeprazole and low CYP2C19 (white bars) refers to an exceptional low formation rate of R-5-hydroxyomeprazole. The Vmax of the cis- and trans-doxepin N-demethylation was significantly lower ($P < 0.05$ and 0.01, respectively) in the CYP2C19 low activity group.

lyze the N-demethylation but only to a minor extent and preferentially of the trans-isomer. From our study it can not be excluded that an isomerization occurs during the N-demethylation of doxepin and that even CYP2C19 might be one of the catalysts of this reaction. The latter might be supported by the fact that the highest portion of cis-desmethyldoxepin was found when rH-CYP2C19 was applied. However, the isomerization was never found *in vitro* using rat or human liver microsomes (4,6) and is thus rather unlikely to play a role in our experimental setting.

Although CYP3A4 was able to N-demethylate at least the trans-isomer of doxepin at the high substrate concentration (Fig. 2), it probably does not play a role under therapeutic conditions. This was further confirmed by the lack of any correlation between the CYP3A4 catalyzed formation of omeprazole sulfone and the doxepin N-demethylation. Although tranylcypromine has been recently described as a potent inhibitor of CYP2A6 (15) there is no evidence at all that CYP2A6 might play a role in the N-demethylation of tricyclic antidepressants. The discrepant result regarding CYP2E1 and the inhibition by phenethyl isothyocanate were probably due to non-specific inhibition of other CYPs, including CYP2C19, CYP1A2, and CYP2C9 at the chosen concentration of 50 μM (16). While contribution of CYP2E1 to the formation of N-desmethyldoxepin is rather unlikely a contribution of CYP2D6 can be excluded. The intrinsic N-demethylation clearance (CL_{int}) calculated after incubation experiments with microsomes from a mix of 6 human livers was more than 4-fold higher for cis-doxepin compared with trans doxepin (36 vs. 8 μl/min).

CONCLUSION

Partly in contrast to a recent publication (6) we found that the N-demethylation of doxepin is mainly catalyzed by CYP2C19, with CYP1A2 and CYP2C9 playing a minor role. At therapeutic concentrations CYP3A4 will not contribute to the N-demethylation of the doxepin isomers but it might play a role at extremely high concentrations. This predominant contribution of CYP2C19 is in perfect agreement to findings with other TCA like imipramine (8).

The intrinsic N-demethylation clearance at therapeutic concentrations is higher for the cis-isomer than for the trans-isomer. This should be regarded as an additional reason for the distortion of the cis to trans ratio of desmethyldoxepin beside the faster metabolism of trans-desmethyldoxepin (4) or a possible isomerization of the trans-isomer during the formation of N-desmethyldoxepin (5).

One should be aware of higher doxepin concentrations in CYP2C19 deficient patients or under co-medication with CYP2C19 inhibitors like fluvoxamine.

ACKNOWLEDGMENTS

This work was supported by a grant (TS 197/01.008/97) of the DPhG (Deutsche Pharmazeutische Gesellschaft) and the Swedish Medical Research Council (3902). Elisabeth Daum-Kreysch is acknowledged for her skillful technical assistance.

REFERENCES

1. R. M. Pinder, R. N. Brogden, T. M. Speight, and G. S. Avery. Doxepin up-to-date; A review of its pharmacological properties and therapeutic efficacy with particular reference to depression. *Drugs* **13**:161–218 (1977).
2. D. C. Hobbs. Distribution and metabolism of doxepin. *Biochem. Pharmacol.* **18**:1942–1954 (1969).
3. K. K. Midha, J. W. Hubbard, G. McKay, E. M. Hawes, E. D. Korchinsky, T. Gurnsey, J. K. Cooper, and R. Schwede. Stereoselective pharmacokinetics of doxepin isomers. *Eur. J. Clin. Pharmacol.* **42**:539–544 (1992).
4. J-H. Yan, J. W. Hubbard, G. McKay, and K. K. Midha. Stereoselective *in vivo* and *in vitro* studies on the metabolism of doxepin and N-desmethyldoxepin. *Xenobiotica* **27**:1245–1257 (1997).
5. H. Ghabrial, C. Prakash, U. G. Tacke, I. A. Blair, and G. R. Wilkinson. Geometric isomerization of doxepin during its N-demethylation in humans. *Drug Metab. Dispos.* **19**:596–600 (1991).
6. V. S. Haritos, H. Ghabrial, J. T. Ahokas, and M. S. Ching. Role of cytochrome P450 2D6 (CYP2D6) in the stereospecific metabolism of E- and Z-doxepin. *Pharmacogenetics* **10**:591–603 (2000).
7. K. Brøsen, E. Skjelbo, B. B. Rasmussen, H. E. Poulsen, and S. Loft. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem. Pharmacol.* **45**:1211–1214 (1993).
8. E. Skjelbo, K. Brøsen, J. Hallas, and L. F. Gram. The mephenytoin oxidation polymorphism is partially responsible for the N-demethylation of imipramine. *Clin. Pharmacol. Ther.* **49**:18–23 (1991).
9. M. Chang, M. L. Dahl, G. Tybring, E. Gotharson, and L. Bertilsson. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics* **5**: 358–363 (1995).
10. T. Friedberg, M. P. Pritchard, M. Bandera, S. P. Hanlon, D. Yao, L. A. McLaughlin, S. Ding, B. Burchell, and C. R. Wolf. Merits and limitations of recombinant models for the study of human P450-mediated drug metabolism and toxicity: an intralaboratory comparison. *Drug Metab. Rev.* **31**:523–544 (1999).
11. J-H. Yan, J. W. Hubbard, G. McKay, and K. K. Midha. Stereoselective and simultaneous measurement of cis- and trans-isomers of doxepin and N-desmethyldoxepin in plasma or urine by high-performance liquid chromatography. *J Chromotogr. B Biomed. Appl.* **691**:131–138 (1997).
12. M. F. Paine, D. D. Shen, K. L. Kunze, J. D. Perkins, C. L. Marsh, J. P. Mc Vicar, D. M. Barr, B. S. Gillies, and K. E. Thummel. First-pass metabolism of midazolam by the human intestine. *Clin. Pharmacol. Ther.* **60**:14–24 (1996).
13. S. Ono, T. Hatanaka, S. Miyazawa, M. Tsutsui, T. Aoyama, F. J. Gonzalez, and T. Satoh. Human liver microsomal diazepam metabolism using cDNA-expressed cytochrome P450s; role of CYP2B6, 2C19 and the 3A subfamily. *Xenobiotica* **26**:1155–1166 (1996).
14. T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, and F. P. Guengerich. Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals; studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**:414–423 (1994).
15. W. Zhang, T. Kilicarslan, R. F. Tyndale, and E. M. Sellers. Evaluation of methoxsalen, tranylcypromine, and tryptamine as specific and selective CYP2A6 inhibitors *in vitro*. *Drug Metab. Dispos.* **29**:897–902 (2001).
16. M. Nakajima, R. Yoshida, N. Shimada, H. Yamazaki, and T. Yokoi. Inhibition and inactivation of human cytochrome P450 isoforms by phenethyl isothiocyanate. *Drug Metab. Dispos.* **29**: 1110–1113 (2001).