Azole Antimycotics Differentially Affect Rifampicin-Induced Pregnan X Receptor-Mediated CYP3A4 Gene Expression

Lucie Svecova, Radim Vrzal, Ladislav Burysek, Eva Anzenbacherova, Lukas Cerveny, Jiri Grim, Frantisek Trejtnar, Jiri Kunes, Milan Pour, Frantisek Staud, Pavel Anzenbacher, Zdenek Dvorak, and Petr Pavek

Departments of Pharmacology and Toxicology (L.S., F.T., F.S., P.P.) and Organic and Inorganic Chemistry (J.K., M.P.), Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic; Departments of Medical Chemistry and Biochemistry (R.V., E.A., Z.D.) and Pharmacology (P.A.), Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic; Centre of Advanced Studies, Faculty of Military Health Sciences, University of Defence in Hradec Kralove (L.C.), Department of Oncology and Radiotherapy, Faculty Hospital in Hradec Kralove, Czech Republic (J.G.); and Gen-Trend, s.r.o. Ceske Budejovice, Czech Republic (L.B.)

Received August 16, 2007; accepted November 7, 2007

ABSTRACT:

Azole antifungal drug ketoconazole has recently been demonstrated as an inhibitor of a ligand-induced pregnane X receptor (PXR)-mediated transcriptional regulation of the CYP3A4 gene through disruption of PXR interaction with steroid receptor coactivator (SRC)-1. In contrast, other clotrimazole-derived antifungal agents are known as potent inducers of CYP3A4 through PXR. In the present study, we examined effects of azole antimitcotics clotrimazole, ketoconazole, econazole, oxiconazole, miconazole, fluconazole, and itraconazole on PXR-mediated expression of CYP3A4. We investigated individual effects of the tested azoles as well as their action on rifampicin-induced PXR-mediated transactivation and expression of CYP3A4 in LS174T cell line and primary human hepatocytes, their interactions with PXR ligand-binding domain, and azole-mediated recruitment of SRC-1 to PXR. In addition, applying the pharmacodynamic approach and dose-response analysis, we aimed to describe the nature of potential interactions of tested azole antimitycotics coadministered with a prototypical PXR ligand rifampicin in transactivation of CYP3A4 gene. We describe additive and antagonistic interactions of partial and full agonists of PXR nuclear receptor in the therapeutic group of azole antimitycotics in rifampicin-mediated transactivation of CYP3A4. We show that oxiconazole is a highly efficacious activator of CYP3A4 transactivation, which could be antagonized by rifampicin in a competitive manner. In addition, we show that activation of the CYP3A4 promoter is a complex process, which is not exclusively determined by azole-PXR interactions, and we suggest that the ability of some azoles to affect recruitment of SRC-1 to PXR modulates their net effects in transactivation of CYP3A4 both in the absence or presence of rifampicin.

Drug-induced expression of CYP3A4 gene is predominantly regulated through the pregnane X receptor (PXR, steroid and xenobiotic receptor, hPXR, nuclear receptor 1I2), a member of the nuclear receptor family. PXR, an important component of the body’s adaptive system, plays a central role in the clearance of xenobiotics. PXR signaling mechanism involves transcriptional regulation of the CYP3A4 gene through complex interaction with steroid receptor coactivator (SRC)-1 (NCOA1) (Bertilsson et al., 1998; Lehmann et al., 1998; Synold et al., 2001; Kliwer, 2003). PXR activity is also influenced by other corepressor proteins, and recruitment of coactivators and the general transcription machinery (Kliwer et al., 1998; Lehmann et al., 1998; Synold et al., 2001; Kliwer, 2003).

Interactions of several azole antifungals with human PXR have recently been investigated. Clotrimazole, known inducer of CYP3A4 expression, and its analogs have been found as highly potent ligands of human PXR, which promotes interaction of PXR with steroid receptor coactivator (SRC)-1 (NCOA1) (Bertilsson et al., 1998; Lehmann et al., 1998; El-Sankary et al., 2001; El-Sankary et al., 2001; Kliwer, 2003; and others). Itraconazole and ketoconazole are weak inducers of CYP3A4 via PXR (Sinz et al., 2006; Huang et al., 2007). However, ketoconazole was reported to inhibit ligand-induced PXR-mediated transcriptional regulation of CYP3A4 and MDRI genes (Takeshita et al., 2002; retinoic acid receptor α (RXRα), binding of the PXR/RXRα heterodimer to response elements (REs) of target genes, release of corepressor proteins, and recruitment of coactivators and the general transcription machinery (Kliwer et al., 1998; Lehmann et al., 1998; Synold et al., 2001; Kliwer, 2003).

Interactions of several azole antifungals with human PXR have recently been investigated. Clotrimazole, known inducer of CYP3A4 expression, and its analogs have been found as highly potent ligands of human PXR, which promotes interaction of PXR with steroid receptor coactivator (SRC)-1 (NCOA1) (Bertilsson et al., 1998; Lehmann et al., 1998; El-Sankary et al., 2001; El-Sankary et al., 2001; Kliwer, 2003; and others). Itraconazole and ketoconazole are weak inducers of CYP3A4 via PXR (Sinz et al., 2006; Huang et al., 2007). However, ketoconazole was reported to inhibit ligand-induced PXR-mediated transcriptional regulation of CYP3A4 and MDRI genes (Takeshita et al., 2002; retinoic acid receptor α (RXRα), binding of the PXR/RXRα heterodimer to response elements (REs) of target genes, release of corepressor proteins, and recruitment of coactivators and the general transcription machinery (Kliwer et al., 1998; Lehmann et al., 1998; Synold et al., 2001; Kliwer, 2003).

ABBREVIATIONS: PXR, pregnane X receptor; DDI, drug-drug interaction; RXRα, retinoid X receptor α (9-cis retinoic acid receptor-α); RE, response element; SRC, steroid receptor coactivator; RT, reverse transcriptase; PCR, polymerase chain reaction; LBD, ligand-binding domain; FBS, fetal bovine serum; SR12813, tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethyl-1,1-bisphosphonate; DMSO, dimethyl sulfoxide; ER, everted repeat; CAR, constitutive androstane receptor; HNF, hepatocyte nuclear factor; GR, glucocorticoid receptor; DR, direct repeat; RID, receptor-interacting domain; VDR, vitamin D receptor; CYP, cytochrome P450; GAL4, yeast transcriptional activator of galactose-metabolizing enzymes.
DMSO (Sigma-Aldrich). The final concentration of DMSO in culture media was 10% (v/v), and the medium was supplemented with 1% fetal bovine serum (FBS). In addition, medium of HepG2 human-derived cell lines that have inducible the CYP3A4 gene reporter plasmid in transactivation assays (Wang et al., 2007).

In the present study, we focused on effects ofazole antymycotics clotrimazole, ketoconazole, econazole, miconazole, fluconazole, and itraconazole on PXR-mediated expression of CYP3A4.

We investigated potency of the individual azole antymycotics to regulate levels of CYP3A4 mRNA in LS174T cells and primary human hepatocytes employing real-time RT-PCR, transactivate CYP3A4 promoter in gene reporter assays, bind to the PXR ligand-binding domain (LBD), and recruit SRC-1 coactivator to PXR in mammalian two-hybrid assays. Furthermore, we examined effects of tested azoles on rifampicin-induced CYP3A4 transcription through PXR. Finally, we aimed to describe the nature of potential pharmacodynamic interactions of tested azole antymycotics coadministered with a prototypical PXR ligand rifampicin in transactivation of the CYP3A4 gene.

We reveal differential effects of several azole antymycotics on PXR-mediated transactivation of CYP3A4, binding to PXR LBD, and recruitment of SRC-1 to PXR. We show additive and antagonistic effects of azole antymycotics on rifampicin-induced PXR-mediated transactivation of CYP3A4. We demonstrate that oxiconazole is one of the most potent inducers of CYP3A4 gene expression via PXR described so far, whose effect in transactivation of CYP3A4 could be competitively antagonized by rifampicin. We also show that ability of some azoles to affect recruitment of SRC-1 to PXR modulates their net effects in transactivation of CYP3A4, both in the absence or presence of rifampicin. We thus suggest that PXR-mediated activation of CYP3A4 promoter is a complex process, whose magnitude is not exclusively determined by binding characteristics of ligands to PXR LBD, and indicate important modulatory role of SRC-1 in PXR-mediated transactivation of CYP3A4.

Materials and Methods

Cell Lines. The human Caucasian hepatocyte carcinoma (HepG2), human colon adenocarcinoma (LS174T), and African Green monkey kidney fibroblast (CV-1) cell lines were purchased from the European Collection of Cell Cultures (Salisbury, UK) and were used within 20 passages after delivery and maintained in antibiotic-free Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS). In addition, medium of HepG2 cells was supplemented with 1% sodium pyruvate and 1% nonessential amino acids (Sigma-Aldrich, St. Louis, MO).

LS174T human colonic epithelial tumor cell line is one of very few human-derived cell lines that can induce the CYP3A4 gene and relatively high expression of PXR (Burk et al., 2005; Cerveny et al., 2007). CV-1 cell line was used since it does not express any hepatocyte-specific transcriptional factors.

Chemicals. Rifampicin, charcoal, SR12813, and cell culture media were purchased from Sigma-Aldrich. Phenol red-free media were purchased from Invitrogen (Carlsbad, CA). FBS was purchased from PAA (Pasching, Austria).

Azole antymycotics were obtained in high-grade pharmaceutical quality from Sigma-Aldrich (clotrimazole); Janssen Pharmaceutica N.V., Beerse, Belgium (ketoconazole, batch E3401; miconazole nitrate, batch I3201); Janssen Research Foundation (itraconazole, batch A201); Hoffmann La Roche, Basel, Switzerland (oxiconazole nitrate, LOT 48182); Cilag AG, Schaffhausen, Switzerland (econazole nitrate, batch 94P4279); and Zentiva, Prague, Czech Republic (fluconazole). Stock solutions (1000× or 3000×) were prepared in DMSO (Sigma-Aldrich). The final concentration of DMSO in culture media was 0.1% (v/v) in all experiments.

Plasmids. A chimeric p3A4-luc reporter construct containing the basal promoter (~362/+53) with the proximal PXR response element (ER6) and the distal xenobiotic responsive enhancer module (~7836/~7208) of the CYP3A4 gene 5'-flanking region inserted to pGL3-Basic reporter vector (Promega, Madison, WI) was described by Goodwin et al. (1999). The expression plasmid for PXR receptor, pSG5-hPXR, was kindly provided by Dr. S. Kliewer (University of Texas, Dallas, TX). The human CAR expression plasmid pCR3-hCAR was kindly provided by Dr. M. Negishi (National Institute of Environmental Health Sciences, Research Triangle Park, NC). The expression plasmid pSG5-hRXRα encoding human RXRα cDNA was a generous gift from Dr. C. Carlberg (University of Kuopio, Kuopio, Finland), pCDNA3-HNF4α expression plasmid was kindly donated by Dr. B. Laine (INSERM Unit 459, Lille, France). The expression plasmid encoding human GRα (pSG5-hGRα) was a generous gift from Dr. J. Palmvio (University of Helsinki, Helsinki, Finland). pRL-TK was purchased from Promega. To construct pDR3-luc and pER6-luc plasmids, we synthesized complementary pairs of oligonucleotides containing three tandem copies of either DR3 or ER6 REs of the CYP3A4 promoter (5′-CTAGCGAAGTACACGTGACCCTCTTGCGATGAGAGAGGTCAGTGGATGGAATGAACTCAAAGGAGGTCAG-3′ to produce pDR3-luc and 5′-CTAGCGAAGTACACGTGACCCTCTTGCGATGAGAGAGGTCAGTGGATGGAATGAACTCAAAGGAGGTCAGTGGATGGAATGAACTCAAAGGAGGTCAGTGGATGGAATGAACTCAAAGGAGGTCAG-3′ to produce pER6-luc). The underlined sequence indicates DR3 or ER6 REs. Oligonucleotides were annealed and cloned into the Nhel- and BglII-digested sites of the pG4L.23 vector containing a minimal promoter (Promega).

Transient Transfection and Luciferase Gene Reporter Assays. Transient transfection assays were carried out using Lipofectamine2000 reagent (Invitrogen) according to the manufacturer’s instruction. HepG2 cells (2 × 10⁴) were seeded into 48-well plates and transfected with a luciferase reporter construct (200 or 300 ng/well), the expression plasmid encoding PXR (50 ng/well), and Renilla reniformis luciferase transfection control plasmid (pRL-TK) 24 h later (30 ng/well). Cells were maintained in phenol red-free medium (200 µl) supplemented with 10% charcoal/dextran-tripanned FBS with azole antymycotics or combinations of drugs for 8 or 24 h.

In the case of transient transfection experiments with pDR3-luc and pER6-luc reporter plasmids, HepG2 cells were cotransfected with 200 ng/well of a reporter plasmid, the expression plasmid encoding PXR (200 ng/well), and pRL-TK (30 ng/well). Subsequently, cells were maintained in phenol red-free medium (200 µl) supplemented with 10% charcoal/dextran-tripanned FBS with azole antymycotics (10 µM) for 48 h. For mammalian two-hybrid assays, we used the mammalian two-hybrid fusion plasmids GAL4-PXR-LBD and VP16-SRC-1-receptor-interacting domain (RID), which were a generous gift from Dr. A. Tahkisata (Teranonom Hospital, Tokyo, Japan; Takahita et al., 2002). The GAL4 fusion plasmid contains the LBD of human PXR (107–434 amino acids) fused to GAL4-LBD (yeast DNA-binding domain), and the VP16 fusion plasmid contains the RID (595–780 amino acids) of SRC-1 fused to Herpes simplex virus VP16 activation domain. In mammalian two-hybrid system, the pG5luc reporter vector, which contains five GAL4 binding sites upstream of a minimal TATA box and the firefly lucerase gene, was purchased from Promega. The pG4L (20 ng/well) and pVP16 (50 ng/well) fusion constructs were transfected along with pG5luc vector (120 ng/well) and pRL-TK (20 ng/well) into HepG2 cells. One day after transfection, azole antymycotics (20 µM) or their combinations with rifampicin (10 µM) were added into media for 8 or 24 h.

In the case of reporter experiments with pG5Luc and pG4L-PXR-LBD plasmids (one-hybrid trans-activation assay in CV-1 cells), 150 ng/well pG5Luc plasmid and 100 ng/well GAL4-PXR-LBD plasmid were used together with 30 ng of pRL-TK. In the assay, an agonist binding to PXR-LBD is detectable through GAL4 activation of the pG5Luc reporter vector. Cells were then exposed to azole antymycotics for 24 h. Luminescence activity was determined with a Genios Plus luminometer (Tecan, Grödig, Austria) in cell lysate using a commercially available lucerase detection system (Dual Luciferase Reporter Assay Kit; Promega).

Real-Time RT-PCR Analysis. Total RNA was isolated from LS174T cells and primary human hepatocytes treated with tested azole antymycotics or their combinations with rifampicin for 24 or 48 h. Total RNA isolation and real-time RT-PCR for CYP3A4 gene were performed as described before (Cerveny et al., 2007). The primer sequences for CYP3A4 expression analysis were the following: reverse primer, 5′-CTG TGA TGC CGA ACA ACT CC-3′; and forward
primer, 5'-CCC AGC CTG CTC ATA GGT TC-3'. The hypoxanthine-guanine phosphoribosyl transferase gene was used as a housekeeping gene (Cerveny et al., 2007). PCR conditions specific for each gene were optimized with respect to MgCl2 concentration and annealing temperature. All samples were run in triplicate simultaneously with negative controls. Melting curve analyses were performed in each real-time RT-PCR experiment. Pfaffl's (2001) method was applied for relative quantification of gene expression normalized to a housekeeping gene. The results are expressed as -fold induction versus control vehicle-treated cells.

Primary Cultures of Human Hepatocytes. Hepatocytes were prepared from lobectomy segments, resected from adult patients for medical reasons unrelated to our research program. Human tissue was obtained according to protocols complying with the current Czech legislation. Human liver samples used in this study were obtained from two patients: LH 18 (woman, 69 years) and LH 19 (woman, 46 years) (Cerveny et al., 2007). Hepatocytes were isolated and cultured as previously described (Cerveny et al., 2007). Following isolation, the cells were plated on collagen-coated culture dishes at a density 1.4 \times 10^5 \text{cells/cm}^2. In addition, two cultures of the long-term human hepatocytes in monolayer batch HEp220216 (77-year-old Caucasian female with hepatic lesion from adencarcinoma, nonsmoker) and HEp220221 (73-year-old Caucasian male with hepatocellular carcinoma, nonsmoker) purchased from Biopredict International (Rennes, France) were used (Meneses-Lorente et al., 2007). The medium was exchanged for serum-free medium the day after delivery, and the cultures were allowed to stabilize for an additional 48 to 72 h prior to treatments. Cultures were maintained at 37°C and 5% CO2 in a humidified incubator. The level of CYP3A4 mRNA expression was analyzed using real-time RT-PCR according to the protocol mentioned above after 24-h treatment with indicated compounds or their combinations.

Cell Viability Test. Cytotoxicity of azole antimycotics after 24, 48, and 72 h of incubation with tested drugs was tested by a colorimetric tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay according to the standard protocol (Roche Diagnostics, Basel, Switzerland). Simultaneously, morphology of cells in culture was microscopically assessed.

Statistical Analyses. One-way analysis of variance followed by Dunnett's multiple comparison post hoc test or Student's t-test was used for statistical analysis of differences between two experimental groups using GraphPad Prism software (GraphPad Software Inc., San Diego, CA). EC50 (xenobiotic concentration required to achieve half-maximum promoter activation) and lmax (representing the overall maximal calculated induction produced by tested compound; maximal responding capacity; maximal efficacy) values were determined according to Hill's equation by nonlinear regression analysis using GraphPad Prism Software (GraphPad Software Inc.) from at least seven-point curves performed in triplicate. Linear regression analyses, slope, and the p value (F test) calculation were also performed employing GraphPad Prism Software.

Results

Induction of CYP3A4 and PXR Gene Expression by Tested Azole Antimycotics in LS174T Cell Line and Primary Human Hepatocytes. First, we tested effects of selected azole antifungals on expression of CYP3A4 mRNA in human intestinal LS174T cells and primary human hepatocytes employing real-time RT-PCR analysis. Transcriptional regulation of the target gene via PXR is well documented (Lehmann et al., 1998; Goodwin et al., 1999). In parallel with CYP3A4 mRNA level, expression of PXR mRNA was also examined.

As shown in Fig. 1A, CYP3A4 mRNA levels were strongly induced by oxiconazole (25-fold), clotrimazole (19-fold), rifampicin (16-fold), miconazole (9-fold), and econazole (9-fold) when compared with control in LS174T cells (n = 3). Interestingly, ketoconazole, oxiconazole, and miconazole significantly (p < 0.05) downregulated PXR mRNA in LS174T cells (Fig. 1A).

In agreement, we found that oxiconazole, miconazole, and econazole are inducers of CYP3A4 mRNA in primary human hepatocytes (Fig. 1B). Contrary to results in LS174T cells, oxiconazole and rifampicin elicited comparable potency to induce CYP3A4 mRNA.

Transactivation of the CYP3A4 Promoter by Azole Antimycotics. Potency of selected azoles to transactivate CYP3A4 gene reporter construct by full-length PXR was assayed in transient transfection experiments in HepG2 cells. We found that oxiconazole, clotrimazole, miconazole, and econazole efficiently activate CYP3A4 promoter through PXR after 8-h incubation in HepG2 cells (Figs. 2A and 3A).
Dose-response analysis using nonlinear regression analysis revealed that the xenobiotic concentration required to achieve half-maximum activation of \(CYP3A4\) reporter plasmid (EC\(_{50}\)) for oxiconazole was approximately 6.1 \(\mu M\) and calculated maximal -fold activation value (\(I_{\text{max}}\)) was 21.9. Rifampicin activated the reported construct with the EC\(_{50}\) value of 1.6 \(\mu M\) and \(I_{\text{max}}\) of 12.4. Thus, dose-response analysis showed that rifampicin is not able to produce full activation of \(CYP3A4\) reporter plasmid under the used experimental conditions. Oxiconazole was also more potent to activate \(p3A4-luc\) reporter after 24-h exposure in HepG2 cells in comparison with rifampicin (data in Fig. 4) or another prototypical PXR ligand SR12813 at an equimolar concentration of 10 \(\mu M\) (76.8- and 53.7-fold activation to control, respectively) under the same experimental conditions with 300 ng of \(p3A4\)-luc and 50 ng of \(pS\)G5-PXR cotransfected per well.

Estimated \(I_{\text{max}}\) for econazole (14.7) was higher than in the case of rifampicin (12.4), clotrimazole (13.9), and miconazole (11.9). EC\(_{50}\) values of econazole and miconazole were higher in comparison with rifampicin (5.6, 12.3, and 1.6 \(\mu M\), respectively) (Fig. 2A). Ketoconazole had a minor effect on \(CYP3A4\) promoter activation; itraconazole and fluconazole did not yield any significant effect after 8 or 24 h of incubation in HepG2 cells (Fig. 3A, dose-response curves are not presented).

**Identification of Oxiconazole, Miconazole, and Econazole as PXR Ligands.** In next experiments, we investigated interactions of tested azole antimycotics with PXR ligand-binding domain. Consistent with the results obtained using the full-length PXR and \(p3A4\)-luc reporter plasmid, clotrimazole, oxiconazole, econazole, and miconazole efficiently activated GAL4 reporter plasmid through GAL4 PXR LBD fusion construct (Fig. 2B). Interestingly, we observed the opposite pattern of activation in the case of rifampicin and oxiconazole in the experiments. Oxiconazole activated the reporter plasmid with higher affinity to PXR LBD than rifampicin (EC\(_{50}\) = 2.9 and 5.6 \(\mu M\), respectively), whereas maximal activation \(I_{\text{max}}\) did not differ significantly between these two compounds (3.2 versus 3.4). Econazole and miconazole activated GAL4 reporter plasmid through GAL4 PXR LBD with the EC\(_{50}\) values of 3.6 and \(>15\) \(\mu M\) and maximal efficacy \(I_{\text{max}}\) values of 2.4 and 2.3, respectively (Fig. 2B). EC\(_{50}\) for miconazole is presented as estimate because miconazole did not reach plateau in the experiments. Clotrimazole was the most potent ligand of PXR in our experiments (EC\(_{50}\) = 2.5 \(\mu M\), \(I_{\text{max}}\) = 5.1) (Fig. 2B). Surprisingly, we found that ketoconazole dose dependently inhibited the GAL4 reporter plasmid activation through GAL4 PXR LBD construct with an EC\(_{50}\) of 17.9 \(\mu M\) and \(I_{\text{min}}\) of 0.25 (Fig. 2B). Fluconazole and itraconazole did not activate the reporter plasmid, suggesting no interaction of the compound with PXR LBD at the tested concentrations (dose-response data not shown; Fig. 3B).

Hence, we conclude that oxiconazole, clotrimazole, econazole, and econazole, but not ketoconazole, itraconazole, and fluconazole efficiently activate \(CYP3A4\) promoter through interaction with PXR LBD (Fig. 2A). We suggest that clotrimazole is a full agonist of PXR, which is able to produce maximal effect in interaction with GAL4-PXR LBD (Fig. 2B). On the other hand, rifampicin, econazole, econazole, and miconazole are partial agonists on PXR LBD, which interact with GAL4-PXR LBD with substantial affinity; however, they do not produce maximal activation (Fig. 2B). Interestingly, we confirmed that ketoconazole induces \(CYP3A4\) mRNA and transactivates \(CYP3A4\) promoter, although it is not an agonist of PXR (Huang et al., 2007).

In the next experiments, we addressed two questions arising from the data. First, we studied how azole antimycotics affect rifampicin-induced activation of \(CYP3A4\) promoter. We supposed to observe additive, synergistic, or antagonistic pharmacodynamic interactions due to different characteristics of tested azoles to transactivate the \(CYP3A4\) promoter via PXR and differential effects of the compounds on SCR-1 recruitment to PXR. Second, we aimed to explain why oxiconazole activates the \(CYP3A4\) promoter with higher efficacy in comparison with rifampicin, although they have comparable \(I_{\text{max}}\) in experiments with the GAL4-PXR LBD plasmid.

**Transactivation of the CYP3A4 Promoter by Combinations of Azole Antimycotics with Rifampicin.** In the next transient transfection experiments with the \(p3A4\)-luc reporter plasmid, we observed that the effect of oxiconazole to transactivate the \(p3A4\)-luc reporter plasmid was significantly \((p < 0.05)\) reduced by rifampicin at concentration of 5 \(\mu M\) or higher after 8-h coincubation in HepG2 cells (Figs. 3A and 4). We suppose that rifampicin attenuates competitively the effect of oxiconazole, which has higher maximal efficacy (\(I_{\text{max}}\))
were treated with the indicated combinations of azoles antimycotics (20 μM) and p3A4-luc reporter plasmid (300 ng/well) and pRL-TK (30 ng/well). HepG2 cells performed with full-length PXR expression plasmid (50 ng/well) together with of SRC-1 coactivator. A, transient transfection experiments in HepG2 cells were performed with pG5luc GAL4 luciferase reporter plasmid, GAL4-PXR LBD, VP16-SRC-1-RID, and pRL-TK plasmids. Cells were then treated with 20 μM azole antimycotics in the presence or absence of rifampicin for 8 h. B, CV-1 cells were transiently transfected with pG5luc GAL4 reporter plasmid (150 ng/well), and pRL-TK transfection control vector. We did not observe any statistically significant additive or antagonist interactions of azole antimycotics in combination with rifampicin, except for ketoconazole (Fig. 3B). In the presence of ketoconazole, we observed significant (p < 0.05) suppression of pG5luc reporter vector activity in CV-1 cells either in the absence or presence of rifampicin (Fig. 3B). These findings contradict to the hypothesis by Huang et al. (2007), who suggested that ketoconazole likely competes with ligands in PXR LBD. Itraconazole and fluconazole neither bound nor affected significantly binding of rifampicin to PXR LBD.

Interactions of Azole Antimycotics with the Ligand-Binding Domain of PXR. Next, we investigated binding of azole antimycotics in combination with rifampicin on PXR ligand-binding domain using the GAL4-PXR LBD fusion construct and pG5luc GAL4 reporter vector. We did not observe any statistically significant additive or antagonist interactions of azole antimycotics in combination with rifampicin on the activation of the pG5luc reporter vector through GAL4-PXR LBD except ketoconazole (Fig. 3B).

Azole Antimycotics Promote PXR-SRC-1 Coactivator Interaction. Interaction of PXR with coactivators is a critical part of the nuclear receptor signaling (Rosenfeld et al., 2006). Recently, ketoconazole was shown to disrupt the interaction of PXR with SRC-1 resulting in suppression of ligand-induced PXR-mediated induction of CYP3A4 and MDR1 genes (Takeshita et al., 2002; Huang et al., 2007; Wang et al., 2007).

We used the mammalian two-hybrid assay to evaluate whether tested azoles individually or in combination with rifampicin (10 μM) affect interaction of PXR with SRC-1. Consistent with previous reports (Lehmann et al., 1998; Synold et al., 2001; Takeshita et al., 2002; Huang et al., 2007, and others), rifampicin and clotrimazole significantly (p < 0.01) promoted the specific interaction of SRC-1 with PXR after 24-h incubation in HepG2 cells (Fig. 3C). In agreement with gene reporter data (Fig. 2A), the effect of rifampicin plateaued from the concentration of 10 μM (data not shown). Oxiconazole and clotrimazole had stronger effects on interaction of PXR or absence of rifampicin at the concentration of 10 μM for 24 h. Data are expressed as mean ± S.D. of a representative experiment performed in triplicate and are shown as -fold induction of normalized luciferase activity relative to solvent (0.1% DMSO) control. Similar profiles were observed in three independent experiments. *p < 0.05.
with SRC-1 in comparison with the effect of rifampicin after 24 h of incubation (Fig. 3C). Econazole and miconazole also yield significant recruitment of SRC-1 to PXR (p < 0.05). In agreement with reporter experiments with the p3A4-luc plasmid (Fig. 3A), rifampicin significantly (p < 0.05) suppressed oxiconazole-mediated interaction of PXR with SRC-1 (Fig. 3C). Fluconazole and itraconazole had no effects on recruitment of SRC-1 to PXR in the absence of rifampicin (Fig. 3C). However, we observed that itraconazole augmented rifampicin-mediated recruitment of SRC-1 to PXR after 8- and 24-h coincubation in HepG2 cells. The simulative effect of itraconazole (20 μM) was statistically significant (p < 0.01) after 8-h coincubation (7.7 ± 0.4-fold activation in comparison with the effect of rifampicin alone, -4.3 ± 0.1-fold activation), but not after 24-h cotreatment (p < 0.07) (data for 24 h in Fig. 3C). Ketoconazole (20 μM) significantly inhibited rifampicin-induced interaction of PXR and SRC-1 after 24-h incubation, which correlates with recent reports (Takeshita et al., 2002; Huang et al., 2007). Interestingly, we did not see additive effects of econazole and miconazole in combination with rifampicin on SRC-1 recruitment (Fig. 3, A and C). We thus hypothesize that additional factor (coactivator/corepressor) might underlie the interactions of the azoles with rifampicin in transactivation of CYP3A4.

Based on the data in Fig. 3, we can conclude that oxiconazole, clotrimazole, miconazole, and econazole are agonists of PXR, which transactivate CYP3A4 promoter and promote SRC-1 coactivator recruitment. In addition, we indicate that oxiconazole promotes recruitment of SRC-1 to PXR more efficiently than rifampicin and clotrimazole, which consequently results in greater transactivation of CYP3A4 promoter by the azole antifungal. Itraconazole stimulated activation of CYP3A4 promoter and recruitment of SRC-1 to PXR, although it did not interact with PXR LBD. Ketoconazole suppresses rifampicin-mediated CYP3A4 transactivation, SRC-1 recruitment to PXR as well as interaction of rifampicin with PXR LBD. Fluconazole did not bind to PXR LBD and had no effect on transactivation of CYP3A4 promoter.

Oxiconazole Competes with Rifampicin in Activation of the CYP3A4 Promoter in Transient Transfection Experiments. To study interaction of rifampicin and oxiconazole in activation of the CYP3A4 promoter, we performed transient transfection experiments in HepG2 cells cultivated for 24 h with increasing concentration of oxiconazole and fixed concentration of rifampicin (and vice versa). As shown in Fig. 4A, rifampicin activated p3A4-luc in a dose-dependent manner and plateaued with the -fold induction of normalized luciferase activity (firefly/Renilla) activity relative to control (0.1% DMSO), *p < 0.05, as compared with cells treated with only 10 μM oxiconazole. #p < 0.05, as compared with cells treated with only 10 μM rifampicin; ##, p < 0.05, as compared with cells treated with 20 μM oxiconazole.

The effects of increasing concentrations of oxiconazole on activation of p3A4-luc in HepG2 cells exposed to a fixed concentration of rifampicin (10 μM) (Fig. 4B). Oxiconazole alone activated p3A4-luc in a dose-dependent manner, with the maximal activation at the concentration of 20 μM after 24-h treatment (Fig. 4B). Combination of 10 μM rifampicin with oxiconazole (20 μM) resulted in significant (p < 0.05) additive increase of p3A4-luc activation in comparison with the individual effect of 10 μM rifampicin (Fig. 4B). However, the activation by the combination of oxiconazole and rifampicin was significantly lower (p < 0.05) than the activation mediated by 20 μM oxiconazole alone (Fig. 4B). Thus, we demonstrate antagonistic effect of rifampicin on oxiconazole-mediated activation of CYP3A4 promoter. These results confirm our hypothesis that rifampicin and oxiconazole compete for activation of the CYP3A4 promoter.

Additive Interactions of Econazole and Miconazole with Rifampicin in Activation of the CYP3A4 Promoter in Transient Transfection Experiments. To study in detail interactions of rifampicin with econazole and miconazole (see Fig. 3A), we performed transient transfection experiments in HepG2 cells treated for 8 h with increasing concentration of rifampicin and fixed concentration of econazole and miconazole (20 μM). We expected that concentrations closed to ECso, of rifampicin (about 1.6 μM) might reduce CYP3A4 promoter activation caused by econazole and miconazole due to different ECso of the compounds (Fig. 2A). In a higher concentration (>5 μM), we supposed additive interactions of rifampicin in combination with econazole or miconazole since these drugs individually activate p3A4-luc reporter plasmid (Fig. 2A).

We found additive effects of both econazole and miconazole in combination with rifampicin in the range of concentrations from 0.5 up to 10 μM (Fig. 5). The effect was statistically significant (p < 0.05) from the 5 μM concentration of rifampicin. We did not observe...
any clear competitive antagonism by rifampicin in azoles-mediated transactivation of the CYP3A4 reporter plasmid.

**Induction of CYP3A4 mRNA by Combinations of Azole Antimycotics with Rifampicin in LS174T Cells and Primary Human Hepatocytes.** Next, we tested effects of oxiconazole and itraconazole in combination with rifampicin on CYP3A4 mRNA expression employing real-time RT-PCR in LS174T cell line and/or in primary human hepatocytes. We did not detect any statistically significant effect of oxiconazole (10 μM) on rifampicin-mediated induction (10 μM) up-regulation of CYP3A4 mRNA in LS174T cells after 48 h of coincubation (22.77 ± 9.22 for rifampicin versus 26.29 ± 12.82 for rifampicin-oxiconazole combination; three independent experiments performed in triplicate). Similarly, we did not find out significantly different induction of CYP3A4 mRNA after treatment of commercial primary human hepatocytes (batch 220221) with combination of oxiconazole and rifampicin from induction mediated by oxiconazole alone (10 μM) after 24-h exposure (5.79 ± 0.91 for combination versus 4.92 ± 0.51 for oxiconazole alone).

We also examined the hypothesis that itraconazole (5 and 10 μM) stimulates rifampicin-mediated induction of CYP3A4 mRNA in primary human hepatocytes after 48-h coincubation with 10 μM rifampicin as suggested in Fig. 3A. We noted variable effect of itraconazole on rifampicin-mediated induction of CYP3A4 in primary human hepatocytes from three donors (data not shown), which at the moment does not support the hypothesis. Ongoing studies with extensive set of primary hepatocyte cultures should elucidate the effects of the azoles on rifampicin-mediated induction of CYP3A4 mRNA.

**Oxiconazole Activates the CYP3A4 Promoter Specifically through PXR.** To test whether additional nuclear receptors are involved in strong activation of CYP3A4 promoter by oxiconazole, we performed gene reporter experiments with the p3A4-luc reporter plasmid and expression vectors encoding human CAR, VDR, GRα, HNF4α, RXRα, and PXR nuclear receptors, which trans-activate CYP3A4 (Martínez-Jiménez et al., 2007). We observed that oxiconazole (10 μM) stimulated significantly (p < 0.01) the reporter activity only in cotransfection with PXR expression plasmid (Fig. 6). This activation was relatively weak in CV-1 in comparison with hepatoma HepG2 cells (Figs. 2A, 3A, and 4). This finding corresponds with the absence of HNF4α and possibly additional hepatocyte-specific factors in CV-1 cells, which are essential for maximal transactivation of CYP3A4 promoter (Tirona et al., 2003b; Li and Chiang, 2006). Cotransfection of CV-1 cells with PXR, CAR, and VDR expression plasmids also yielded activation of CYP3A4 promoter plasmid in the absence of an exogenous ligand (Fig. 6). This well known phenomenon is likely caused by an endogenous ligand or a ligand-independ-ent activation of CYP3A4 promoter reporter plasmid. Luciferase activity of p3A4-luc was not significantly increased in the absence or presence of oxiconazole in cotransfection with RXRα, GRα, and HNF4α expression plasmids or their empty expression plasmids pcDNA3 (Fig. 6) or pSG5 (data not shown). Interaction of oxiconazole with GRα and CAR has been also examined using pGRE3-luc and p2B6(PBREM)-SV40-luc reporter plasmids with appropriate REs for tested nuclear receptors (Cerveny et al., 2007). These experiments confirm no statistically significant agonistic effect of oxiconazole on GRα and CAR (data not shown). Hence, we can conclude that oxiconazole at the 10 μM concentration had negligible effect on activation of p3A4-luc through CAR, VDR, GRα, HNF4α, and RXRα nuclear receptors.

**Activation of ER6 and DR3 Response Elements by Tested Azole Antimycotics Correlate with Activation of the CYP3A4 Promoter.** Finally, we tested activation of chimera reporter plasmids with replic-ate DR3 and ER6 response elements of CYP3A4 promoter by tested azoles. The ligand-activated PXR forms a heterodimer with RXRs and binds to two central REs of CYP3A4 promoter: the inverted repeat separated by six bases (ER6) located in the proximal promoter and the direct repeat spaced by three bases (DR3) in the distal xenobiotic responsive enhancer module (Bertilsson et al., 1998; Lehmann et al., 1998; Goodwin et al., 1999). DR3 and ER6 REs synergistically transactivate the CYP3A4 gene via PXR, and disruption of the REs destroys 80 to 90% of PXR-mediated responsiveness of CYP3A4 promoter (Goodwin et al., 1999). We supposed that activation of the pDR3-luc and pER6-luc reporter plasmids would correlate with activation of p3A4-luc promoter only on the assumption that no additional cis-acting elements are involved in azole-mediated activation of the CYP3A4 promoter.

We found that activation of the ER6 response element with tested azole antimycotics well correlated with activation of the p3A4-luc reporter (r² = 0.90; p = 0.0003) (Fig. 7A). Similarly, we found...
correlation between pDR3-luc and p3A4-luc activation with tested azole antimycotics ($r^2 = 0.79$; $p = 0.003$; $F$ test), with the exception of itraconazole, which activated pDR3-luc and pER6-luc, but not the p3A4-luc reporter plasmid (Fig. 7B). Rifampicin was a less efficacious activator of both pDR3-luc and pER6-luc reporter plasmids than oxiconazole, miconazole, econazole, and clotrimazole at the concentration of 10 μM under the experimental conditions. Thus, we suggest that oxiconazole activates the CYP3A4 promoter via PXR specifically through binding to DR3 and ER6 REs.

Cell Viability Testing after Treatment with Azole Antimycotics. Several azole antimycotics have been shown to be toxic to HepG2 cells or rat hepatocytes (Somchit et al., 2004; Sinz et al., 2006). Therefore, the cytotoxic potential of all compounds was tested employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay in HepG2 and LS174T cell lines. We found indications of lower viability of HepG2 cells in the case of itraconazole (60%) > oxiconazole (70%) = ketoconazole after 72-h incubation at the 10 μM concentration. Incubation of HepG2 with tested azoles for 24 or 48 h did not affect cellular cultures in comparison with the control. In LS174T cells, we did not observe any changes in cell viability after 24-h incubation, whereas the 48-h incubation with 10 μM itraconazole decreased formation of tetrazolium salt by about 30%. No microscopic changes in cell morphology were observed in HepG2 or LS174T cell lines after 24- and 48-h treatment with any tested compound.

Discussion

In the current paper, we reveal differential effects of several azole antimycotics on rifampicin-induced PXR-mediated transactivation of CYP3A4. We describe additive and antagonistic interactions of selected azole antimycotics with rifampicin in the process. We demonstrate that oxiconazole is one of the most potent inducers of CYP3A4 gene expression via PXR described so far, whose effect in transactivation of CYP3A4 could be competitively inhibited by rifampicin. We also found that econazole and miconazole are potent PXR ligands and inducers of CYP3A4, which in coadministration with rifampicin activate CYP3A4 promoter in an additive manner. Itraconazole stimulated activation of the CYP3A4 promoter and recruitment of SRC-1 to PXR, although it did not interact with PXR LBD. Clotrimazole, a highly potent PXR ligand, neither affected rifampicin-induced transactivation of CYP3A4 nor produced any interaction with rifampicin on PXR LBD. We also show that ketoconazole suppressed rifampicin-mediated CYP3A4 transactivation, SRC-1 recruitment to PXR, as well as interaction of rifampicin with PXR LDB. On the other hand, ketoconazole induced CYP3A4 mRNA through CYP3A4 promoter REs, albeit it is not an agonist of PXR. Fluconazole did not bind to PXR LDB and had no effect on transactivation of CYP3A4 promoter. We thus show differential effects of tested azoles on PXR-controlled CYP3A4 transactivation and suggest that its magnitude is not solely determined by binding characteristics of azoles (or their combinations with rifampicin) to PXR LBD.

The CYP3A4 gene regulation is a complex process mediated by numerous transcription factors (PXR, CAR, CCAAT/enhancer binding protein α, CCAAT/enhancer binding protein β, GRα, HNF4α, and HNF3α) and multiple promoter/enhancer elements (Martínez-Jiménez et al., 2007). Although PXR is a critical determinant of xenobiatics-induced CYP3A4 expression, other trans-acting factors such as HNF4α, SRC-1, SHR, and peroxisome proliferator-activated receptor γ coactivator 1α are essential for maximal transactivation of the CYP3A4 gene (Tirona et al., 2003b; Li and Chiang, 2006). For most tested compounds so far, a good correlation has been observed between transactivation of the CYP3A4 promoter and ligand-PXR binding assay data (Zhu et al., 2004). However, discrepancies were found with some compounds showing high binding affinity in the ligand-binding assay, but with low efficacy to transactivate CYP3A4 (Zhu et al., 2004). In the present study, employing dose-response analysis, we found that oxiconazole produced greater maximal responding capacity ($I_{max}$) to activate CYP3A4 luciferase reporter plasmid in comparison with rifampicin ($I_{max} = 21.9$ versus 12.4; Fig. 2A). Rifampicin activated the CYP3A4 promoter with lower EC50 than oxiconazole (1.6 versus 6.1 μM, respectively; Fig. 2A). In contrast, employing the transactivation assay with GAL4-PXR LBD and pG5Luc plasmids, oxiconazole activated the reporter plasmid with higher affinity to PXR LBD than rifampicin (EC50 = 2.9 and 5.6 μM, respectively), whereas maximal activation did not differ significantly between these two compounds ($I_{max}$ = 3.2 versus 3.4). This discrepancy clearly indicates that interaction with PXR LBD does not directly determine the magnitude and character of CYP3A4 promoter transactivation by oxicon-
azole and rifampicin (Fig. 3). We hypothesize that additional factors such as recruitment of coactivators, release of corepressors from unliganded PXR, suppression of short/small heterodimer partner gene expression, presence of a liver-enriched transcriptional factor, histone-deacetlylase activity, or binding to promoter DNA determine the net effect of PXR ligands to transactivate CYP3A4 promoter.

Dose-response relationships presented in Fig. 2 suggest that rifampicin is not able to produce full activation of p3A4-luc and pG5luc reporter plasmids under the used experimental conditions in comparison with other potent PXR ligands, clotrimazole and oxiconazole (Fig. 2A). In agreement with our data, PXR ligands such as hyperforin, nifedipine, SR12813, reserpin, o,p’-DDT, fenvalerate, pesticide oxadiazole, herbicide pretichlor, steroid 3α-hydroxy-5β-pregnane-11,20-dione methanesulfonate, and several other compounds have been shown to be by about 20 to 70% more potent at equimolar concentrations than rifampicin in transient transfection assays with different reporter plasmids of CYP3A4 gene or in transient transactivation assays with GAL4-PXR LBD (Bertilsson et al., 1998; Moore et al., 2002; Sinz et al., 2006; Lemaire et al., 2007; and others).

In our experiments, oxiconazole was by about 60% more potent than rifampicin after 8-h treatment and almost 2-fold more potent after 24-h treatment at the concentration of 20 μM in the transient transfection experiment with p3A4-luc, and calculated I_{max} for oxiconazole was higher by 77% than I_{max} for rifampicin (Fig. 2A), which rates oxiconazole among the most potent inducer of CYP3A4 via PXR. A drug that produces maximal possible effect through a receptor is referred to as a full agonist, and a drug that displays submaximal effectiveness is referred to as a partial agonist. In pharmacodynamic theory, a partial agonist acts also as an antagonist in the presence of a full agonist. When it binds to the receptor, it also occupies the drug-binding site competitively with respect to a full agonist. A higher concentration of a full agonist will be required to produce a maximal effect. We observed this phenomenon in the case of coadministration of oxiconazole with rifampicin (Figs. 3B and 4). We also demonstrate that econazole and miconazole are potent ligands of PXR, activators of the CYP3A4 promoter, and inducers of CYP3A4 mRNA. Their combinations with rifampicin activate the CYP3A4 promoter in an additive manner since we observed partial summation of their individual effects (Figs. 3A and 5). Interestingly, another potent PXR ligand clotrimazole did not elicit any additive effect with rifampicin on CYP3A4 transactivation or recruitment of SRC-1 to PXR (Fig. 3).

In the current paper, we used HepG2 and CV-1 cell lines, which express no or very low mRNA levels for major uptake and efflux drug transporters, which determine intracellular concentration of drugs and thus their activity in interaction with transcriptional factors (Hilgen-dorf et al., 2007). In contrast, in hepatocytes expressing numerous drug transporters, cellular concentration of a PXR ligand may be affected (Tirona et al., 2003a). Interface of drug transporters and nuclear receptors thus should be considered in the final effect of an inducer in hepatocytes.

During preparation of the paper, Wang et al. (2007) published their data on activation of CYP3A4 (~10,466 to +53)-luc reporter plasmid by oxiconazole, miconazole, and fluconazole in HepG2 cells. These authors suggested that the compounds suppress basal and rifampicin-activated PXR-mediated activation of the plasmid after 48-h incubation. In contrast, in our experiments with highly inducible CYP3A4 reporter plasmid and employing chimera reporter plasmids with ER6 and DR3 REs normalized to pRL-TK R. reniformis control vector, oxiconazole and miconazole appeared to be highly efficacious activators of CYP3A4 promoter after 8-, 24-, or 48-h incubation. In addition, oxiconazole and miconazole promoted recruitment of SRC-1 to PXR in two-hybrid assay and significantly induced CYP3A4 mRNA in LS174T cells and in primary human hepatocytes. Explanation of the discrepancy in not apparent now because both CYP3A4 reporter plasmids and protocols of the transient transfection have been validated in past. Other experimental conditions and approaches should be used to elucidate the conflicting observations.

We suppose that cytotoxicity is another important factor, which can lead to false negative or positive results. Therefore, to minimize any cytotoxicity of selected azole compounds, we designed some transient transfection experiments for 8-h incubation, and we used concentrations of tested drugs up to 20 μM. A shorter incubation period also eliminates potential biotransformation of tested compound, although it is very low in HepG2 cells (Rodriguez-Antona et al., 2002). In addition, 8-h incubation is too short to up-/down-regulate any transcriptional factor or nuclear receptors.

CYP-mediated DDIs are one of the most alarming problems in clinical practice and in the pharmaceutical industry (Lin, 2006). Treatment of serious mycotic infections by systemicazole antifungal agents (itraconazole, fluconazole, ketoconazole, voriconazole) in multimorb patients is associated with the number of severe DDIs. Recent estimates suggest that as many as 95% of hospitalized patients treated with azole antifungals may receive medications capable of producing major or moderate pharmacokinetic interactions (Bates and Yu, 2003).

It is generally known that CYP inhibition is the basis of DDIs mediated by azole antmycotics (Venkatarkrishnan et al., 2000). The inhibitory capability of azoles stems from their mechanism of action, which is inhibition of fungal CYP-mediated synthesis of ergosterol. As a result, the azole antifungals interact also with human cytochrome P450 and cause DDIs with a large number of drug classes, including antineoplastics, steroids, antimicrobials, antiretrovirals, cardiovascular agents, psychotropics, and oral contraceptives (Venkatarkrishnan et al., 2000; Shakeri-Nejad and Stahlmann, 2006). Our results indicate that several azole antmycotics up-regulate CYP3A4 gene expression. Therefore, it is urgent to consider potency of azole drugs to affect gene expression in pharmacotherapy. This could prevent unintended consequences in terms of DDIs but also in metabolism of endogenous compounds. Our current data imply that pharmacodynamic interactions on PXR in transactivation of CYP3A4 gene may result in DDIs at the level of gene regulation. Further studies should elucidate additive and antagonistic interactions of coadministered PXR ligands in CYP3A4 transactivation and study dose-response relationships of clinically relevant PXR ligands.

In conclusion, we describe agonistic and antagonistic pharmacodynamic interactions of partial and full agonists on PXR nuclear receptor in transactivation of the CYP3A4 gene in the therapeutic group ofazole antmycotics. We identify oxiconazole as a highly potent activator of CYP3A4 promoter via PXR and an efficacious inducer of CYP3A4 mRNA. On the contrary, we established rifampicin as a partial agonist of PXR-mediated transactivation of CYP3A4 in transient transfection gene reporter experiments. In addition, we show that activation of CYP3A4 promoter is a complex process determined not solely by azole-PXR LBD interactions and suggest an important modulatory role of SRC-1 coactivator in some azole-mediated CYP3A4 transactivation.

References
Burk O, Arnold KA, Nussler AK, Schaeffeler E, Eilmova E, Avery BA, Avery MA, Fromm MF, and Eichelbaum M (2005) Antimalarial artemisinin drugs induce cytochrome P450 and MDR1...


Address correspondence to: Dr. Petr Pavek, Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Pharmacy, Heyrovského 1203, Hradec Kralove, CZ-500 05, Czech Republic. E-mail: petr.pavek@faf.cuni.cz