Drug Metabolism and Disposition

Supplemental Data

Drug–drug interaction risk assessment of esaxerenone as a perpetrator by in vitro studies, and static and physiologically based pharmacokinetic models

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Supplemental Methods

Inhibitory potential of esaxerenone on P450 isoform activities in human liver microsomes

Incubation

The potential for reversible inhibition and time-dependent inhibition (TDI) of each isoform by esaxerenone was investigated in vitro using pooled human liver microsomes. The reaction mixture for reversible inhibition and TDI contained human liver microsomes (final concentration: 0.2 or 2 mg protein/mL for reversible inhibition and TDI, respectively), 0.05 mM EDTA, 0.025 or 0.1 M Na-K phosphate buffer (for CYP2A6 and others, respectively; pH 7.4), and 0.1–100 μM esaxerenone as well as probe substrate: 30 μM phenacetin for CYP1A2, 1 μM coumarin for CYP2A6, 100 μM bupropion for CYP2B6, 10 μM paclitaxel for CYP2C8, 5 μM diclofenac for CYP2C9, 20 μM (*S*)-mephenytoin for CYP2C19, 10 μM bufuralol for CYP2D6, 40 μM chlorzoxazone for CYP2E1, and 5 μM midazolam, and 50 μM testosterone for CYP3A4. The incubation mixtures were prewarmed at 37°C for 5 min, followed by the addition of the NADPH-generating system (0.5 mM β-$NADP^+$, 5.0 mM glucose-6-phosphate, 5.0 mM $MgCl_2$, and 1.0 U/mL glucose-6-phosphate dehydrogenase) and incubation for the indicated time at 37°C. To investigate the effect of preincubation, the incubation mixtures were prewarmed at 37°C for 5 min, the NADPH-generating system was added, and the incubation mixtures were preincubated at 37°C for 30 min. The probe substrate solution was subsequently added and incubated for the indicated time at 37°C. The reaction was terminated by adding an equal volume of acetonitrile. To calculate inhibition constants (K_i), reaction mixtures were incubated with various concentrations of esaxerenone and probe substrates. To determine the inactivation parameter of CYP3A4, the reaction mixtures were prewarmed at 37°C for 5 min, followed by the addition of the NADPH-generating system. Immediately (0 min) and 4, 8, 12, 20, and 30 min after start of the incubation, an aliquot was taken and diluted 10 times with incubation mixture (EDTA,

Na-K phosphate buffer, and the NADPH-generating system). Each substrate (i.e., 25 μM for midazolam or 250 μM for testosterone) was immediately added to initiate the reaction. After incubation at 37°C for 5 min, the reaction was terminated by adding an equal volume acetonitrile. Each analytical sample was mixed with internal standard solution and centrifuged at 1,600 × *g* at 4°C for 10 min, and the concentrations of the probe substrate metabolites in the supernatant were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

LC-MS/MS analysis

Liquid chromatography was performed using a Waters 2695 or 2795 (Waters, Milford, MA). Chromatographic separation was carried out in the analytical column of a Symmetry C18 (3.5 μm, 2.1 mm I.D. × 100 mm, Waters) with an oven temperature set at 40°C. The autosampler temperature was maintained at 10°C. The mobile phase consisted of two solvents: A (acetonitrile containing 0.1% acetic acid) and B (water containing 0.1% acetic acid) for CYP1A2, CYP2B6, CYP2D6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4; and A (methanol) and B (5 mM ammonium acetate) for others. Separations were achieved by using a gradient of both solvents. Mass spectrometric detection was performed on a Quattro Micro (Waters) with an electrospray ionization (ESI) interface. The desolvation temperature was maintained at 400°C. The quantification was performed according to peak area ratio. Data acquisition and processing were performed using MassLynx 4.0 SP4 (Waters).

Parameter calculation

The metabolic activity of each isoform was calculated using the following equation:

$$\text{Metabolic activity} = \frac{\text{metabolite concentration}}{\text{microsomal protein concentration} \times \text{incubation time}}$$

Remaining activity was calculated using the following equation:

$$\text{Remaining activity} = \frac{\text{metabolic activity with the test compound}}{\text{metabolic activity without the test compound}}$$

If the remaining activity in the presence of 100 μM esaxerenone was ≤50.0%, the remaining activity was analyzed by WinNonlin version 5.2.1 (Certara, Princeton, NJ) pharmacokinetics analysis software to determine the 50% inhibitory concentration (IC_{50}) values using the following equation:

$$E = E_{max} \times \left(1 - \frac{C}{[C + IC_{50}]}\right)$$

where E is remaining activity, E_{max} is maximum remaining activity, and C is esaxerenone concentration. For the calculation of the inhibition constant (K_i), the data were plotted using an Eadie–Hofstee plot, showing metabolic activities (Activity) on the y-axis and the values obtained by dividing the metabolic activities by substrate concentrations (Activity / [S]) on the x-axis. The mode of inhibition was determined based on the results obtained by the Eadie–Hofstee plot. When the mode of inhibition was judged as competitive, the K_i value was calculated by analysis with the following model formula in WinNonlin version 5.2.1:

Competitive inhibition

$$\frac{1}{v} = \frac{K_m}{V_{max} \times [S] \times K_i} \times [I] + \frac{K_m}{V_{max} \times [S]} + \frac{1}{V_{max}}$$

where, v is metabolic activity, V_{max} is the maximum metabolic activity, [S] is substrate concentration, K_m is the Michaelis constant, and [I] is the concentration of esaxerenone. When the mode of inhibition was not determined by the Eadie–Hofstee plot, the K_i value was calculated with the following model formula:

Mixed-type inhibition

$$\frac{1}{v} = \left\{\frac{K_m}{V_{max} \times [S] \times K_i} + \frac{1}{V_{max} \times K_i'}\right\} \times [I] + \frac{K_m}{V_{max} \times [S]} + \frac{1}{V_{max}}$$

Then, the results that showed smaller CV values in WinNonlin were chosen. To calculate inactivation parameters, the natural logarithm of the remaining activity at each concentration of esaxerenone was plotted against the pre-incubation time. The apparent inactivation rate constant (k_{obs}) was determined

from the negative slope of the fitting line from the area showing an initial inhibition rate for esaxerenone concentration. To determine the maximum inactivation rate constants (k_{inact}) and the concentration at half the k_{inact} (K_I) value, the hyperbolic relationship between the k_{obs} values and esaxerenone concentrations ([I]) was fitted to the following equation using WinNonlin:

$$k_{obs} = \frac{k_{inact} \times [I]}{(K_I + [I])}$$

Inhibitory potential of esaxerenone on UGT1A1 and UGT2B7 activities in human liver microsomes

Incubation

The inhibitory potential of esaxerenone on human uridine 5′-diphospho-glucuronosyltransferase (UGT) 1A1 and UGT2B7 activities was investigated in vitro using pooled human liver microsomes. To calculate IC_{50} values, the following reaction mixture was used: 0.05 mg protein/mL human liver microsomes (final concentration), 5 mM UDPGA, 50 mM Tris-HCl buffer (pH 7.5, containing 5 mM $MgCl_2$), 0.1–100 μM esaxerenone, and 25 μg/mL alamethicin as well as 15 μM β-estradiol or 600 μM 3′-azido-3′-deoxythymidine as probe substrate for UGT1A1 or UGT2B7, respectively. The incubation mixtures were pre-warmed at 37°C for 5 min, UDPGA was added, and the mixture was incubated at 37°C for 10 min. The reaction was terminated by mixing with acetonitrile containing β-estradiol 3-β-D-glucuronide-d_3 for UGT1A1 and AZT β-D-glucuronide-d_3 for UGT2B7 as internal standards, and the samples were filtered for deproteination. To calculate the K_i of UGT1A1, 0, 10, 20, 40 or 60 μM esaxerenone was incubated with 7.5, 15, 30, or 45 μM β-estradiol in the reaction mixture as described above. The concentrations of the probe substrate metabolites were measured by LC-MS/MS.

LC-MS/MS analysis

Liquid chromatography was performed using a LC-10ADvp series (Shimadzu, Kyoto, Japan).

Chromatographic separation was carried out in the analytical column of a Synergi Hydro-RP (4 μm, 2 mm I.D. × 100 mm, Phenomenex, Torrance, CA) with an oven temperature set at 40°C and an autosampler temperature of 4°C. The mobile phase consisted of two solvents: A (water containing 0.1% formic acid and 5% acetonitrile) and B (acetonitrile containing 0.1% formic acid). Separation was achieved by using a gradient of both solvents. Mass spectrometric detection was performed on a 4000 QTRAP (SCIEX, Framingham, MA) with an ESI interface. The turbo gas temperature was maintained at 600°C. The quantification was determined according to peak area ratio. Data acquisition and processing were performed by Analyst version 1.4.2 (SCIEX).

Parameter calculations

The metabolic activity and remaining activity of each isoform was calculated as described above. If the remaining activity in the presence of 100 μM esaxerenone was ≤50.0%, the remaining activity was analyzed by WinNonlin to determine the IC_{50} value using the following equation:

$$\text{Remaining activity (\% of control)} = \frac{IC_{50}}{IC_{50} + I} \times 100$$

where I is esaxerenone concentration.

K_i values were determined by WinNonlin using the following equation:

$$E = \frac{V_{max} \times [S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]}$$

where E is the metabolic activity, V_{max} is the maximum value of metabolic activity, [S] is the substrate concentration, K_m is the Michaelis constant, and [I] is the concentration of esaxerenone.

Induction potential of esaxerenone on P450 isoform activities in fresh human hepatocytes

Incubation

The potential of esaxerenone to induce CYP1A2, CYP2B6, and CYP3A4 was investigated using

primary cultures of fresh human hepatocytes from three donors. Approximately 1.5×10^5 hepatocytes/well were cultured at 37°C on 48-well culture plates in a "sandwich" configuration using collagen Type 1, both for coating of the plates and for the culture overlay. The culture medium was hepatocyte-defined medium (Becton Dickinson, Franklin Lakes, NJ) supplemented with 100 U/mL penicillin (Sigma-Aldrich) and 100 μg/mL streptomycin (Sigma-Aldrich). The cells were allowed to attach overnight, during which, 10% (v/v) fetal bovine serum (Invitrogen, Carlsbad, CA) was added to the medium. The medium was subsequently removed, and a second collagen layer was added and allowed to gel for 30 min. Before the start of treatment, the cells were cultivated under serum-free conditions. Incubation with solvent, test, and reference articles was started 4–7 days after seeding. Esaxerenone at a final concentration of 0.1–10 μM for CYP1A2 or 0.1–30 μM for CYP2B6 and CYP3A4 as well as omeprazole, phenobarbital, or rifampicin for CYP1A2, CYP2B6, and CYP3A4, respectively (as reference articles), were added to fresh incubation medium at 3 consecutive days of culture for a total treatment time of 72 h. The determinations were performed in duplicate for CYP1A2 and triplicate for CYP2B6 and CYP3A4. Phenacetin *O*-deethylation, testosterone 6β-hydroxylation, and bupropion hydroxylation were used to specifically determine CYP1A2, CYP2B6, and CYP3A4 enzyme activities, respectively. After the 72-h treatment period, cultured cells were washed with William's E medium and incubated with William's E medium containing phenacetin, testosterone, or bupropion, where appropriate. The incubation medium was mixed with an internal standard solution, and the concentrations of the probe substrate metabolites were determined by LC-MS/MS.

LC-MS/MS analysis

Liquid chromatography was performed using a Prominence UFLC (Shimadzu). Mass spectrometric detection was performed on an API4000 (SCIEX) with an ESI interface. For acetaminophen analysis, chromatographic separation was performed in the analytical column of a Shim-pac XR-ODS C18

column (3.0 × 50 mm, 2.2 μm particle size; Shimadzu) with an oven temperature of 70°C. The mobile phase consisted of two solvents: A (water containing 5% methanol, 5 mM ammonium acetate, and 0.2% formic acid) and B (methanol containing 5% water, 5 mM ammonium acetate, and 0.2% formic acid). Separations were achieved by using a gradient of both solvents. The desolvation temperature was maintained at 600°C. To analyze hydroxybupropion (BUP-OH) and 6β-hydroxytestosterone (6β-OHT), chromatographic separation was performed in the analytical column of an Onyx C18 (3.0 mm I.D., 100 mm length, monolithic; Phenomenex) for BUP-OH and Onyx C18 (3.0 mm I.D., 50 mm length, monolithic; Phenomenex) for 6β-OHT. The column oven temperature was set at 40°C for BUP-OH and 45°C for 6β-OHT. The mobile phases for BUP-OH were as follows: A (water containing 5% methanol, 5 mM ammonium acetate, and 0.2% formic acid) and B (methanol containing 5% water, 5 mM ammonium acetate, and 0.2% formic acid). The mobile phases for 6β-OHT were as follows: A (water containing 5% methanol, 10 mM ammonium acetate, and 0.2% formic acid) and B (methanol containing 10% water, 10 mM ammonium acetate, and 0.2% formic acid). Separations were achieved by using a gradient of both solvents. The interface heater temperature was maintained at 500°C for BUP-OH and 600°C for 6β-OHT. CYP induction was calculated and presented as "fold change", whereby the activity of cells treated with 0.1% (v/v) DMSO served as solvent controls.

Determination of cytotoxicity and mRNA expression

After incubation, the viability of all cell cultures was determined using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Cell cultures were washed twice and incubated with a highly water-soluble tetrazolium salt (WST-8) solution provided with the kit for 120 min. The resultant absorbance caused by formazan dye generated from WST-8 by the activities of dehydrogenases in cells was measured. After incubation with the WST-8 solution, hepatocyte cultures were washed twice with William's E medium and treated with PBS containing 1.5 mg/mL collagenase and 1.5 mM Ca^{2+} for 30 min at 37°C.

Cells were harvested and centrifuged 1000 × *g* for 5 min at 4°C. The supernatants were subsequently discarded, and 50 μL RNA-later (Thermo Fisher Scientific, Waltham, MA) solution was added to each cell pellet. The total RNA was isolated using the MagNA Pure 96 System (Roche Diagnostics, Rotkreuz, Switzerland). Next, cDNA was synthesized from the isolated total RNA using the Omniscript Reverse Transcriptase kit (Qiagen, Hilden, Germany). Then, mRNA was quantified by quantitative reverse transcription polymerase chain reaction using TaqMan technology and ViiA 7 Real-Time PCR (Applied Biosystems, Foster City, CA). The mRNA levels of the test or reference article-treated cells were calculated as the fold change compared to cells treated with dimethyl sulfoxide (DMSO).

Parameter calculation

The induction parameters—the maximum induction effect (E_{max}) and the half-maximum effective concentration (EC_{50})—were calculated by WinNonlin using the following equation:

$$\text{Fold change} = 1 + \frac{E_{max} \times C}{EC_{50} + C}$$

Inhibitory potential of esaxerenone on transporters

The inhibitory effects of esaxerenone on the transport of substrates by the following proteins were examined: human organic anion transporter (OAT) 1, OAT3, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, organic cation transporter (OCT) 1, and OCT2 in transfected MDCK-II cells; human multidrug and toxin extrusion transporter (MATE) 1 and MATE2-K in transfected HEK293 cells; and human BCRP and P-gp in Caco-2 cells.

OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2

MDCK-II cells were maintained in Dulbecco's modified Eagle medium with low glucose, low sodium

bicarbonate, and 10% fetal bovine serum. The cells were seeded at 1.5×10^5 cells/well on 24-well transwell membrane plates (1-μm PET Millicell Cell Culture Plate Assembly and Millicell receiver tray; Millipore, Burlington, MA) and incubated at 37°C in 5% CO_2 for approximately 24 hours. The cells were transfected with a nonviral transient transfection system, and transport assays were performed approximately 24 hours after transfection. Both the apical and basal sides of the wells were washed with Hank's balanced salt solution (HBSS), excess wash solution was aspirated, and 150 μL mineral oil at 37°C was added to the apical well. The cells were preincubated at 37°C for 15 min with HBSS containing 0.1–30 μM esaxerenone in the basal well. After preincubation, the basal buffer was replaced with HBSS containing each substrate—10 μM [^{14}C]metformin for OCT1 and OCT2, 2 μM [^{3}H]p-aminohippurate for OAT1, 0.75 μM [^{3}H]estrone-3-sulfate for OAT3, 2 μM [^{3}H]estradiol-17β-D-glucuronide for OATP1B1, and 10 μM [^{3}H]cholecystokinin-8 for OATP1B3—and esaxerenone, and the cells were subsequently incubated at 37°C for 5 min. All incubations contained 0.5% DMSO and were performed in triplicate. After incubation, the basal side was washed with ice-cold PBS and 200 μL acetonitrile/water (1:1 v/v) was added to the apical side. The cell lysate was mixed adequately, and a 100-μL aliquot of the solution was collected and mixed with 700 μL scintillation fluid. The radioactivity was counted using liquid scintillation counter. The IC_{50} was calculated as follows:

$$V = \frac{V_0}{1 + ([I]/IC_{50})^n}$$

where V_0 is the mean transporter-mediated flux in the absence of esaxerenone, V is the transporter-mediated flux in the presence of esaxerenone throughout the concentration range tested, [I] is the esaxerenone concentration, and n is a Hill coefficient.

BCRP and P-gp

Caco-2 cells were maintained in Dulbecco's modified Eagle medium with high glucose and 10% fetal bovine serum. The cells were seeded at 1.5×10^5 cells/well on 24-well transwell membrane plates.

Transport assays for BCRP and P-gp were performed approximately 7 or 14 days after seeding, respectively. Both the apical and basal sides of wells were washed with HBSS. The cells were preincubated with HBSS containing 0.1–30 μM esaxerenone at 37°C for 30 min. The buffer was subsequently removed and replaced with HBSS containing substrate—25 nM [^{3}H]genistein for BCRP and 100 nM [^{3}H]digoxin for P-gp—as well as the indicated concentrations of esaxerenone for the donor side and HBSS containing the indicated concentrations of esaxerenone for the receiver side. The cells were incubated at 37°C for 120 min, and a 100-μL aliquot of the solution was collected from each receiver well and mixed with 700 μL scintillation fluid. The radioactivity was counted using a liquid scintillation counter. The IC_{50} was calculated using the following equation:

$$P_{app} = \left(\frac{V_r}{A \times C_0}\right) \times \left(\frac{C_r}{\Delta t}\right)$$

where P_{app} is the apparent permeability from the apical-to-basal ($P_{app,A\ to\ B}$) and basal-to-apical directions ($P_{app,B\ to\ A}$) (in cm/s), V_r is the volume of the receiver compartment (in mL), C_0 is the initial concentration of the donor solution, A is the monolayer area (in cm^2), C_r is the receiver well concentration at the end of the incubation, and Δt is the change in time from t = 0 (in s):

$$P_{app}\ \text{ratio} = \frac{P_{app,B\ to\ A}}{P_{app,A\ to\ B}}$$

$$V = \frac{V_0}{1 + ([I]/IC_{50})^n}$$

where V_0 is the mean (P_{app} ratio − 1) in the absence of esaxerenone, V is the (P_{app} ratio − 1) in the presence of esaxerenone throughout the concentration range tested, [I] is esaxerenone concentration, and n is a Hill coefficient.

MATE1 and MATE2-K

HEK293 cells expressing MATE1 were seeded in 24-well plates coated with collagen I (Corning, Corning, NY) at approximately 2.5×10^5 cells/well and incubated at 37°C in a 5% CO_2 incubator for

2 days. Meanwhile, HEK293 cells were seeded in 24-well plates coated with collagen I at 1.5×10^5 cells/well and incubated at 37°C in a 5% CO_2 incubator for 1 day. After incubation, the cells were transfected with vectors containing MATE2-K cDNA or vector only, and these transfected cells were incubated at 37°C in a 5% CO_2 incubator for 2 days. The cells were subsequently washed with 1 mL HBSS-NH_4Cl, 300 μL HBSS-NH_4Cl–containing DMSO (0.2%) was added, and the cells were preincubated at 37°C for 15 min. After preincubation, the entire preincubation solution was removed, 300 μL HBSS containing substrate ([^{14}C]metformin, 10 μM) with or without 0.3–100 μM esaxerenone was added, and the cells were incubated at 37°C for 1 min for MATE1 or 2 min for MATE2-K. The concentration of DMSO was 0.2% in all incubation conditions, and all incubations were performed in triplicate. After incubation, the assay solution was removed and the cells were rinsed once with 1 mL ice-cold PBS containing 0.2% bovine serum albumin twice with 1 mL ice-cold PBS. After the PBS was completely removed, the cells were lysed in 0.5 mL 0.1 M NaOH. The cell lysates were mixed adequately by pipetting, and a 300-μL aliquot of the solution was collected in a polypropylene vial. Scintillator solution (5 mL) was added to each vial, and the radioactivity was counted using a liquid scintillation counter. The protein content was assayed using a BCA protein assay kit (Thermo Fisher Scientific). The IC_{50} was calculated as follows:

$$\text{Uptake clearance} = \frac{\text{Uptake amount}}{\text{Protein content} \times \text{Initial concentration} \times \text{Incubation time}}$$

$$\text{Remaining activity (\% of control)} = \frac{\text{D} - \text{C}}{\text{B} - \text{A}} \times 100$$

where A is the uptake clearance into control cells in the absence of esaxerenone, B is the uptake clearance into transporter expressing cells in the absence of esaxerenone, C is the uptake clearance into control cells in the presence of esaxerenone, D is the uptake clearance into transporter expressing cells in the presence of esaxerenone.

$$\text{Remaining activity (\% of control)} = \frac{\text{IC}_{50}}{\text{IC}_{50} + \text{I}} \times 100$$

Supplemental Table 1 Input parameters of midazolam in GastroPlus simulation

Parameters (units)			
Molecular weight	325.77	V_c (L/kg)	0.326
logP	3.25[a]	K_{12} (1/h)	1.57
$f_{u,p}$ (%)	3.1[a] (2.32[c])	K_{21} (1/h)	1.05
R_b	0.55[a]	CYP3A_K_m (μg/mL)	0.896[d]
P_{eff} (cm/s × 10^{-4})	6.73[a]	CYP3A_$V_{max, liver}$ (mg/s)	0.373[d]
Solubility (mg/mL)	0.082[b]	CYP3A_$V_{max, gut}$ (mg/s)	0.184[e]

logP, octanol-water partition coefficient; $f_{u,p}$, unbound fraction in plasma; R_b, blood/plasma concentration ratio; P_{eff}, effective permeability.

[a] Gertz et al., 2011, *Drug Metab Dispos* **39**:1633–1642

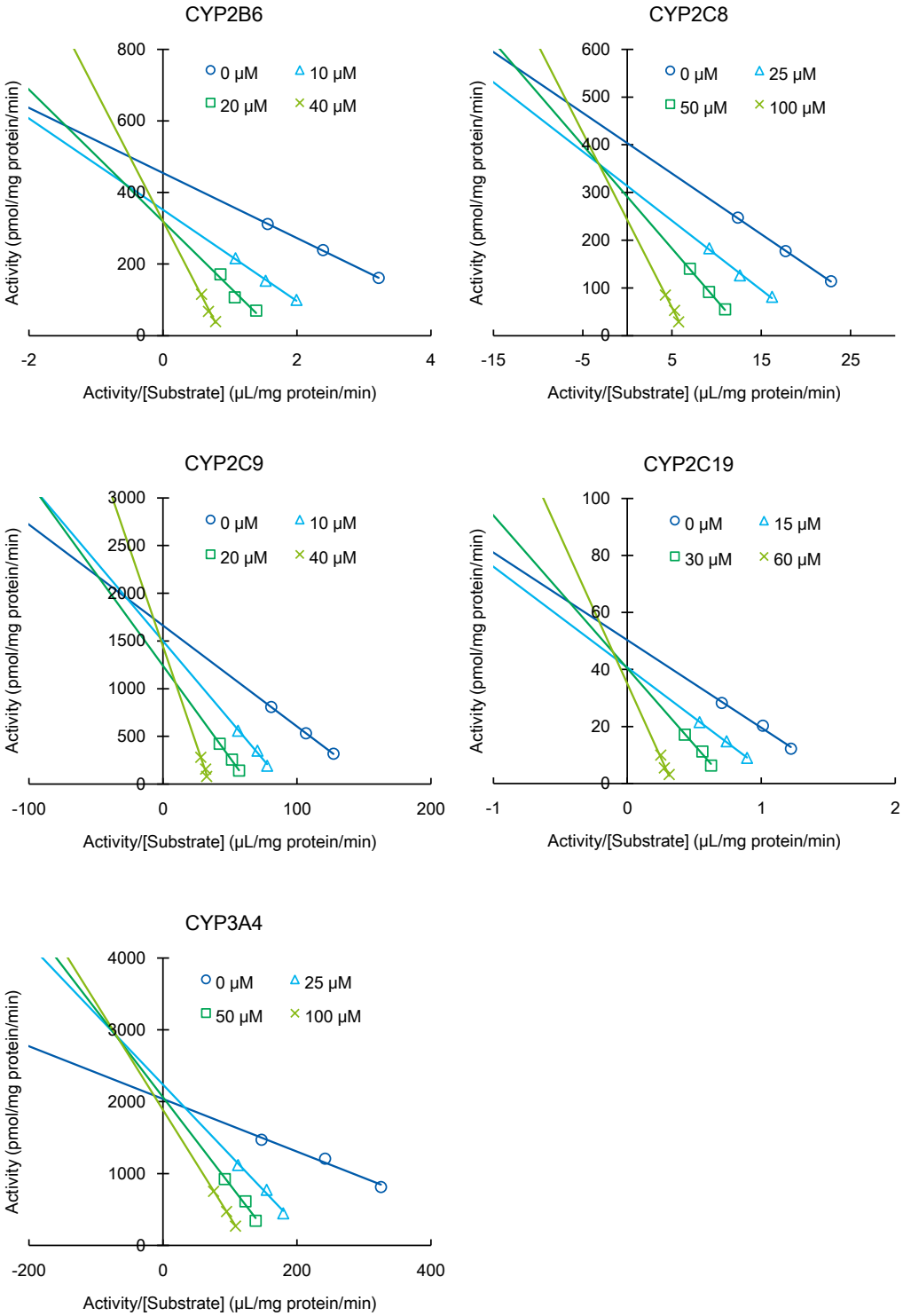
[b] pH 6.39 (Andersin, 1991, *J Pharm Biomed Anal* **9**:451–455)

[c] Adjusted by GastroPlus for possible binding to plasma lipids

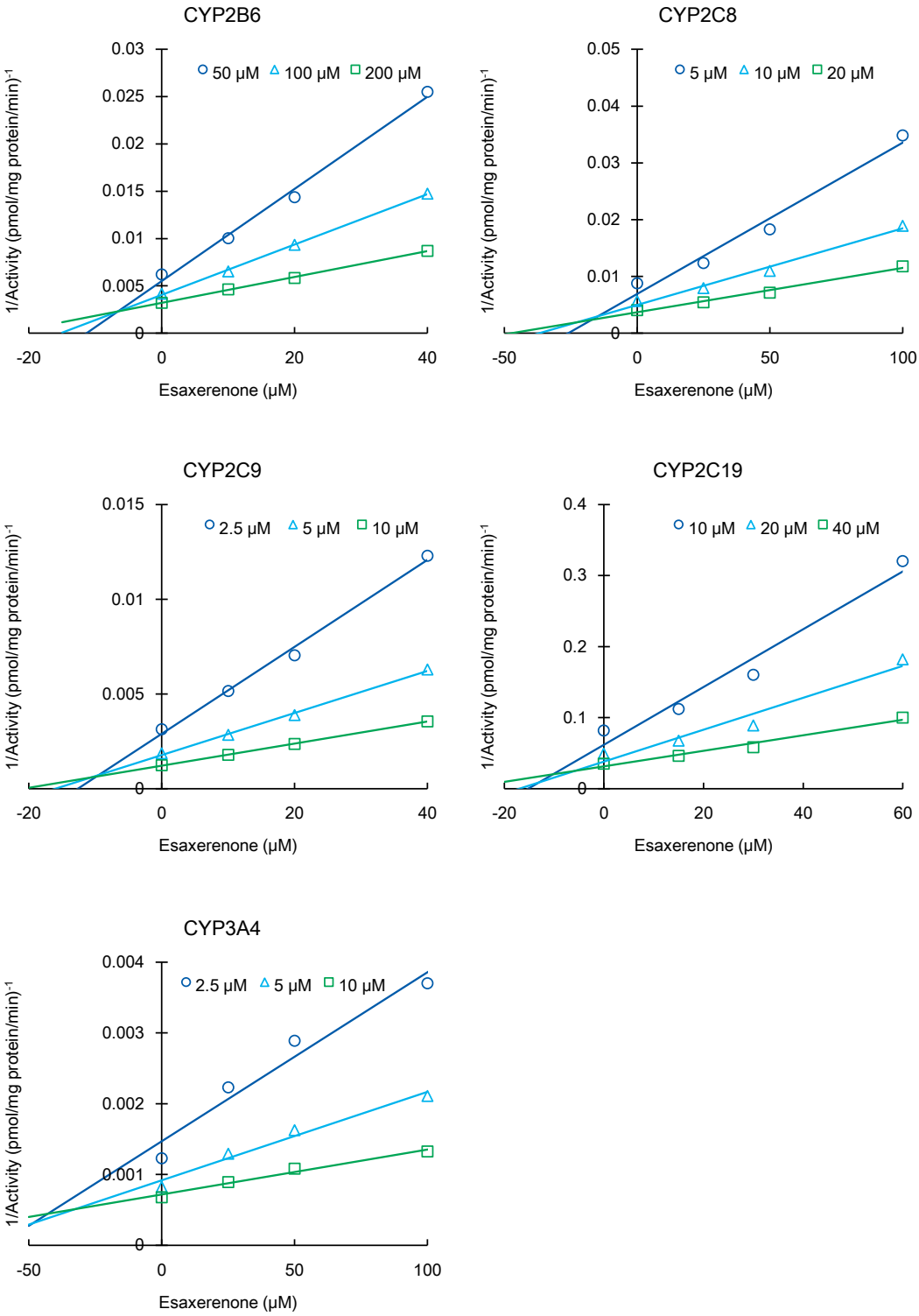
[d] Converted using the unit converter in GastroPlus (Thummel et al., 1996, *Clin Pharmacol Ther* **59**:491–502)

[e] Adjusted to achieve the observed F_g (Yamada et al., 2020, *Drug Metab Dispos* **48**:288-296)

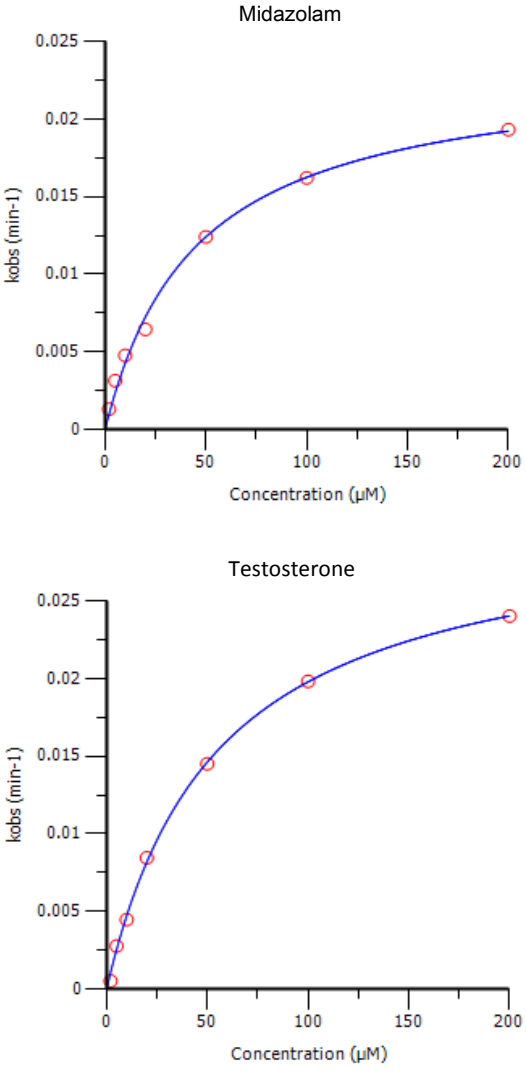
Supplemental Fig. 1. Reversible inhibition by esaxerenone on CYP isoforms in human liver microsomes (Eadie–Hofstee plot).



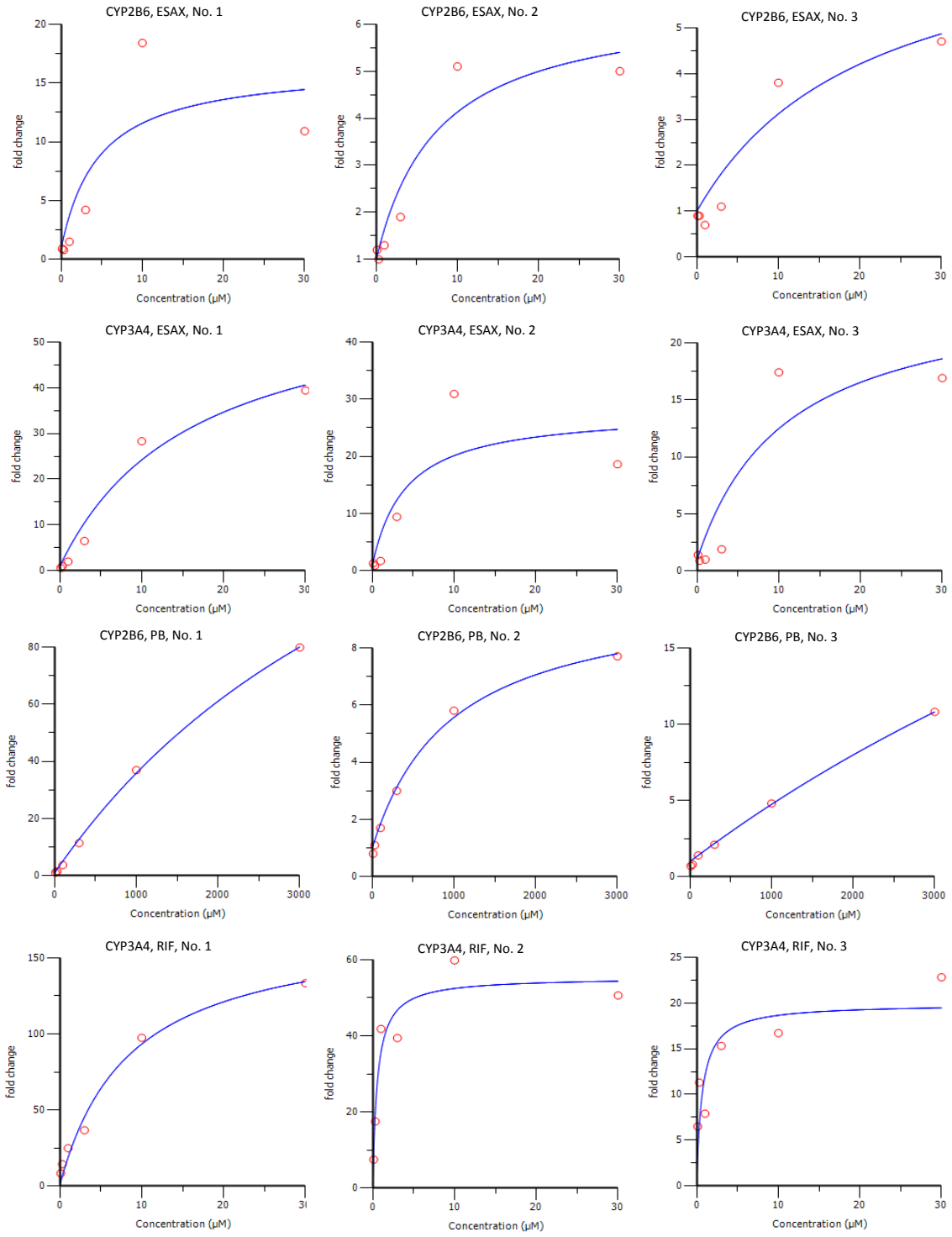
Supplemental Fig. 2. Reversible inhibition by esaxerenone on CYP isoforms in human liver microsomes (Dixon plot).



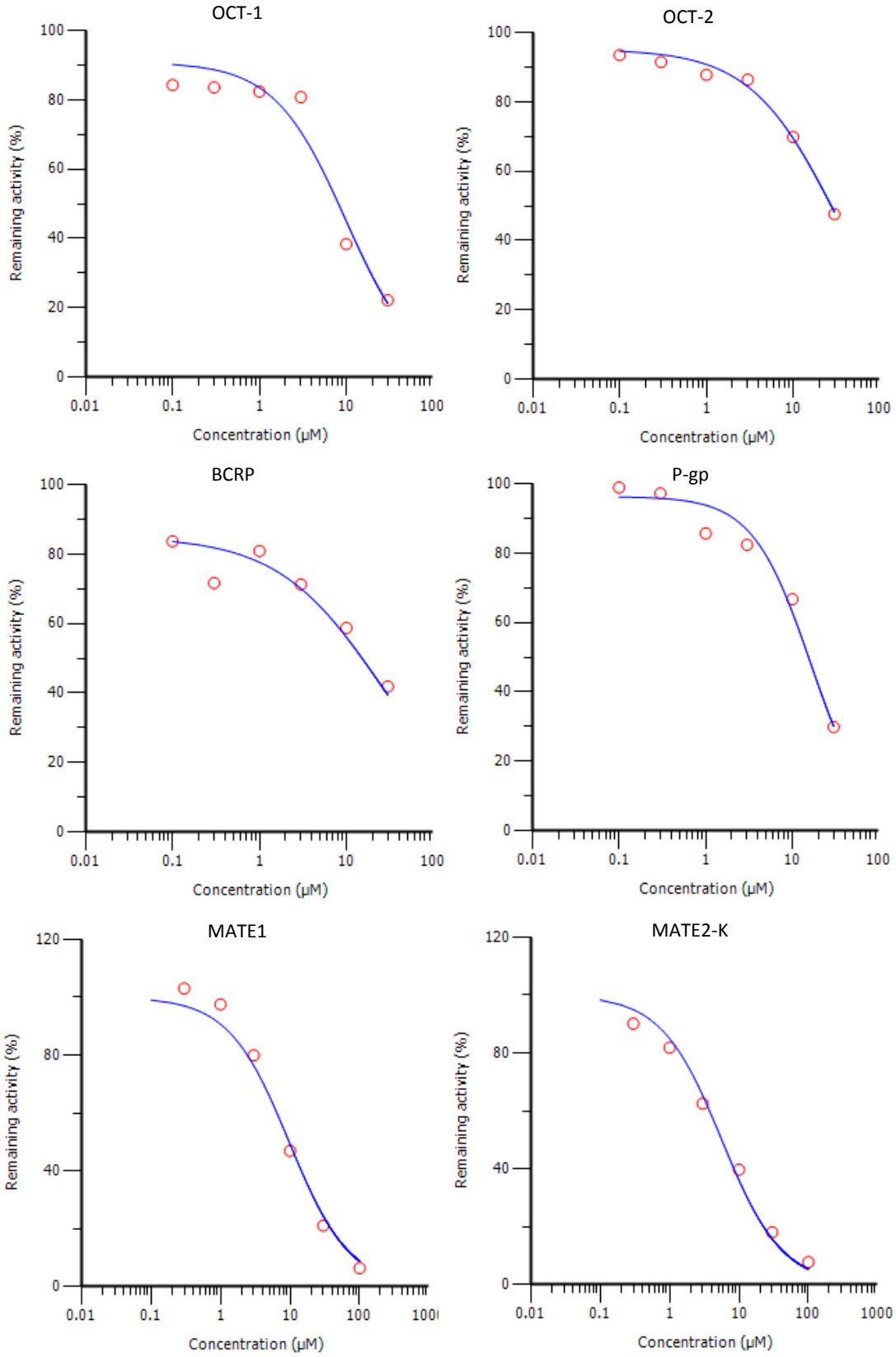
Supplemental Fig. 3. Time-dependent inhibition by esaxerenone on CYP3A in human liver microsomes. The x-axis represents concentrations of esaxerenone, and the y-axis represents the observed apparent inactivation rate constant, k_{obs}.



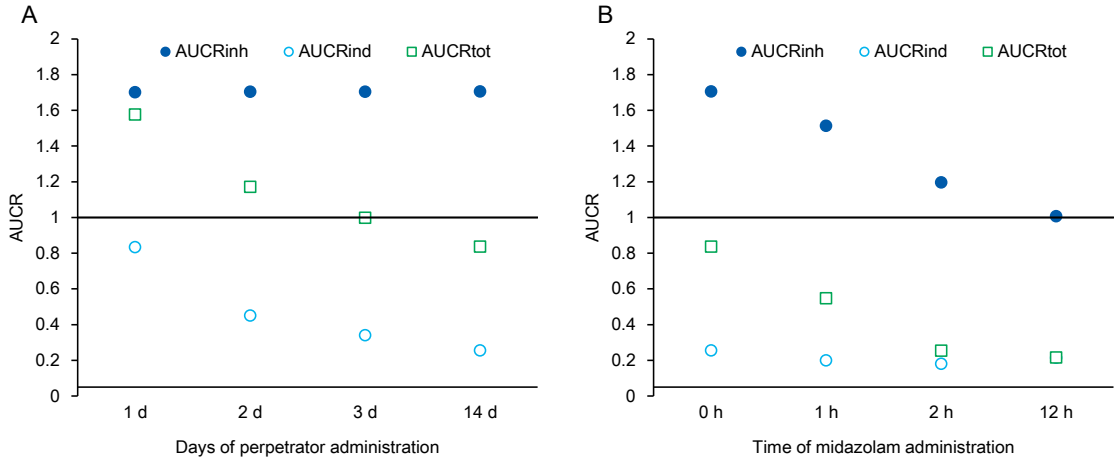
Supplemental Fig. 4. Induction by esaxerenone (ESAX), phenobarbital (PB), and rifampicin (RIF) on CYP2B6 or CYP3A4 in human hepatocytes.



Supplemental Fig. 5. Inhibition by esaxerenone on major transporters in their expressing cells.

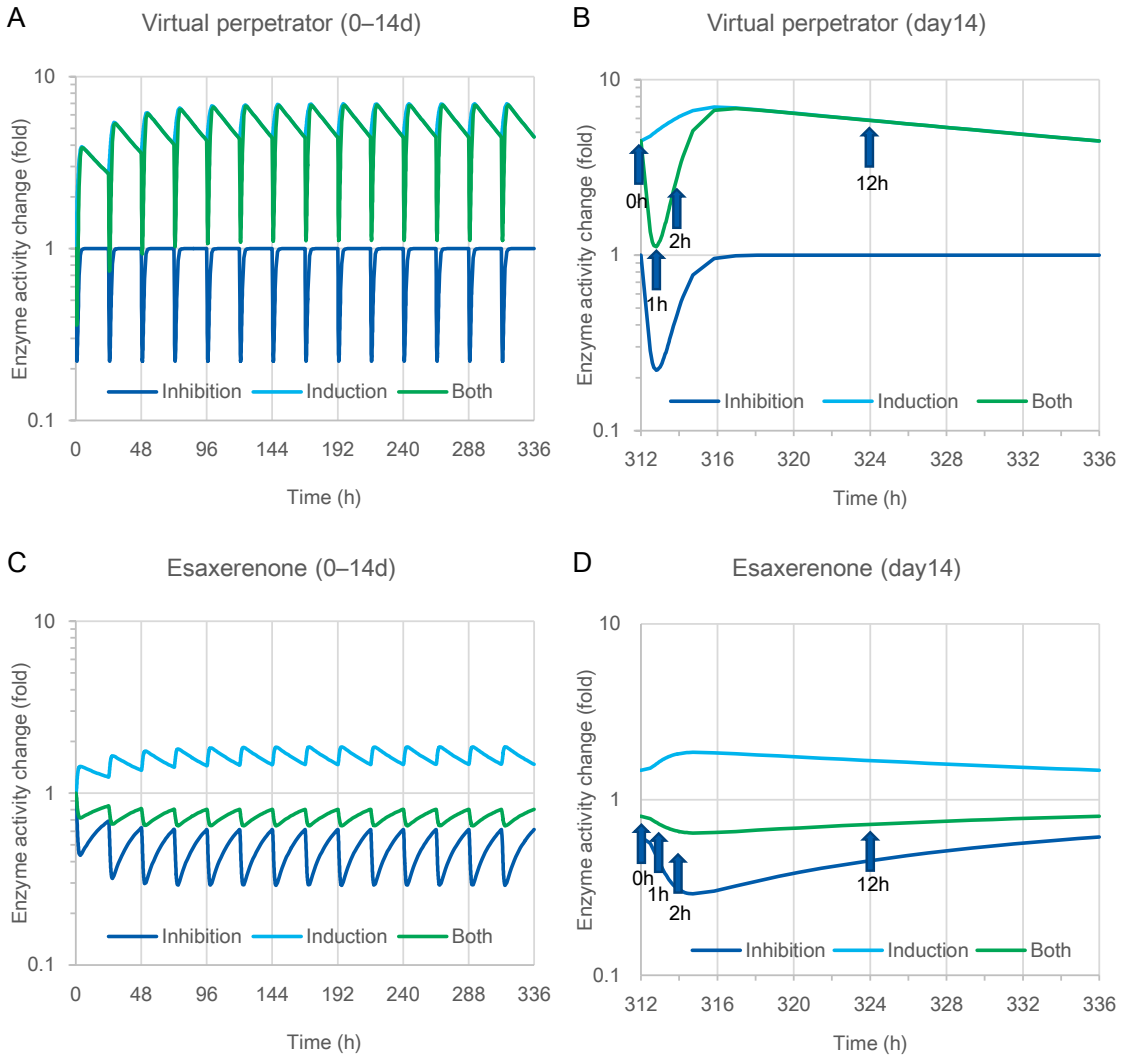


Supplemental Fig. 6. Simulated midazolam AUCRs when a virtual perpetrator was co-administered with different perpetrator administration durations (A) and midazolam administration timing after perpetrator administration (B).



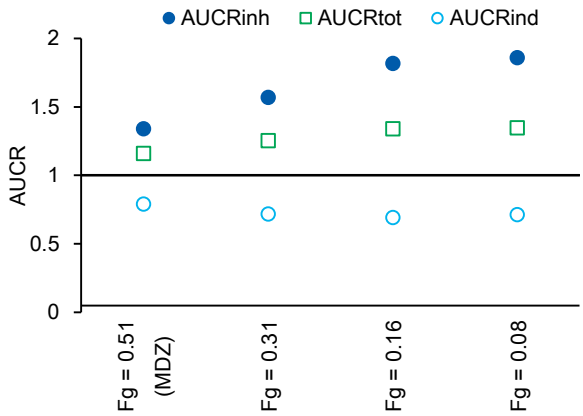
The DDIs of midazolam and a virtual perpetrator were simulated with reversible inhibition only ($AUCR_{inh}$), induction only ($AUCR_{ind}$), or both inhibition and induction ($AUCR_{tot}$) with different perpetrator administration duration (A) and midazolam administration timing (B). The virtual perpetrator had only reversible inhibition and induction (no TDI) with a K_i and EC_{50} $1/50^{th}$ those of esaxerenone.

Supplemental Fig. 7. Enzyme activity–time profile in the enterocytes.



The change of the amount of active enzyme in jejunum compartment 1 (as an example) was simulated by GastroPlus and plotted. As the change of activity by reversible inhibition cannot be calculated by GastroPlus, it was calculated by Microsoft Excel for the virtual perpetrator with the simulated jejunum compartment 1 concentration using the following formula: 1 / (1 + [I] / Ki). The changes of activity by both inhibition and induction were calculated by multiplying the activity change by inhibition and induction for the virtual compound. The arrows indicate the timing of midazolam administration after the dose of esaxerenone. Because reversible inhibition is transient, DDIs are strongly affected by the timing of substrate administration. Time-dependent inhibition and induction are less variable. Supplemental Fig. 7A and B correspond to Supplemental Fig. 6, and Supplemental Fig. 7C and D correspond to Fig. 4.

Supplemental Fig. 8. Simulated AUCRs of virtual substrates when esaxerenone was co-administered.



The DDIs of virtual substrates and esaxerenone were simulated with inhibition only ($AUCR_{inh}$), induction only ($AUCR_{ind}$), or both inhibition and induction ($AUCR_{tot}$). The virtual substrates were created by increasing the CYP3A_$V_{max,\ gut}$ of the midazolam (MDZ) model by 2, 4, or 8 times, resulting in F_g values of 0.31, 0.16, and 0.08, respectively. Then the DDIs with esaxerenone (5 mg for 14 days) were simulated. When the substrate F_g was smaller, $AUCR_{inh}$ was larger. However, because the $AUCR_{ind}$ was smaller, the change of the $AUCR_{tot}$ that incorporated both inhibition and induction was small.