Ziritaxestat Drug–Drug Interaction with Oral Contraceptives: Role of SULT1E1 Inhibition

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In vitro signals indicate that ziritaxestat is a weak cytochrome P450 (CYP) 3A4 inhibitor and inducer. Therefore, potential drug–drug interactions (DDIs) with oral contraceptives were examined at a time when ziritaxestat was under development for treatment of fibrotic diseases. This open-label, crossover (fixed sequence) DDI study enrolled healthy, nonpregnant women aged 18–65 years (n = 15) who were using highly effective contraception, such as a nonhormonal intrauterine device, bilateral tubal occlusion, or sexual abstinence. A single dose of oral contraceptive (0.03 mg ethinyl estradiol (EE) and 3 mg drospirenone (DRSP)) was administered on days 1, 8, and 18, and ziritaxestat 600 mg once daily was administered from days 8 to 23. Co-administration resulted in a 2.8-fold and 2.4-fold increase in EE maximum plasma concentration (C_{max}) and area under the plasma drug concentration–time curve from time zero to infinity (AUC_{0-inf}), respectively (day 18 vs. day 1). DRSP C_{max} and AUC_{0-inf} increased by 1.1-fold and 1.2-fold, respectively. DRSP is a CYP3A4 substrate, meaning increased EE exposure with ziritaxestat was not due to CYP3A4 inhibition. Ziritaxestat inhibition of EE glucuronidation and sulfation was quantified by liquid chromatography with tandem mass spectrometry in day 1 and day 18 plasma samples after EE conjugate hydrolysis. The ratio of EE AUC from time of administration up to the time of the last quantifiable concentration (AUC_{last}) with/without hydrolysis by arylsulfatase was substantially lower on day 18 vs. day 1, suggesting ziritaxestat is a potent inhibitor of sulfation; EE glucuronidation was largely unaffected by ziritaxestat. *In vitro* assessment confirmed ziritaxestat is a potent inhibitor of sulfotransferase family 1E member 1 (half-maximal inhibitory concentration < 0.8 μM). These findings highlight the importance of assessing enzymes other than CYP3A4 when investigating potential DDIs with oral contraceptives.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ The US Food and Drug Administration (FDA) Guidance for Industry regarding oral contraceptive drug–drug interaction (DDI) studies recognizes that, whereas progestins are predominantly metabolized by CYP3A4, ethinyl estradiol (EE) is metabolized by multiple enzymes, particularly phase II enzymes. This has implications for oral contraceptive DDIs with investigational drugs, but data are currently lacking in the clinical setting.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We investigated the role of enzymes other than CYP3A4 in the clearance of EE, following an oral contraceptive DDI study that indicated ziritaxestat substantially increased EE exposure via an alternative route.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ *In vivo* and *in vitro* investigations revealed that EE exposure increased upon co-administration with ziritaxestat via potent inhibition of SULT1E1. To our knowledge, this is the first time the effect of a potent inhibitor of SULT1E1 on EE clearance has been reported in a clinical setting.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Our findings underscore the need to extend the FDA guidance for oral contraceptive DDI investigations to include assessment of UDP-glucuronosyltransferase and sulfotransferase.

Due to their frequent use as birth control, oral contraceptives—generally in the form of a fixed combination of estrogen and progestins—are often co-administered with a variety of therapies. The metabolism of oral contraceptives, such as progestins (e.g., drospirenone (DRSP) and norethisterone) and estrogens (e.g., ethinyl estradiol (EE)), is not completely understood owing to their use becoming widespread before the advent of systematic clinical development programs.[1] Characterization of potential drug–drug

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interactions (DDIs) with oral contraceptives is important; induction of metabolism may lead to instances of unplanned pregnancy, whereas inhibition of metabolism could increase EE exposure to doses equivalent to ≥ 50 µg, which may be associated with an increased risk of venous thromboembolism.[2] The US Food and Drug Administration (FDA) Guidance for Industry recommends oral contraceptive DDI studies be performed if an investigational drug is an inhibitor or inducer of cytochrome P450 (CYP) 3A4 or has teratogenic effects.[2] Moreover, this guidance recognizes that, whereas progestins are predominantly metabolized by CYP3A4, EE is metabolized by multiple enzymes, although their relative contributions to EE metabolism remain unclear.[2] Co-administration of strong CYP3A4 inhibitors, such as voriconazole and ketoconazole, only moderately increase the systemic exposure of EE (by 60 and 40%, respectively), suggesting that CYP3A4-mediated oxidation is not predominant in its metabolism.[1] By contrast, strong CYP3A4 inhibition results in a significant and consistent increase in DRSP exposure (100–170% with boceprevir and ketoconazole, respectively).[1]

Ziritaxestat (GLPG1690) is an autotaxin inhibitor that was, until recently, in development for the oral treatment of fibrotic diseases, such as idiopathic pulmonary fibrosis (phase III clinical trial registration numbers: NCT03711162 and NCT03733444) and systemic sclerosis (phase IIa clinical trial registration number: NCT03798366). *In vitro* studies indicate that ziritaxestat is a weak inducer and inhibitor of CYP3A4.[3] Predictions based on the *in vitro* data indicated that ziritaxestat-mediated induction of CYP3A4 is expected to decrease exposure of a CYP3A4 probe substrate by ≤ 30%, with inhibition expected to result in a maximum increase in probe substrate metabolism of 1.5-fold. The inhibition of CYP3A4 was competitive and reversible (data on file). These characteristics make it necessary to evaluate any pharmacokinetic (PK) DDIs between ziritaxestat and oral contraceptives that could adversely alter their efficacy and/or safety.

The present study was designed to evaluate the effect of single and multiple oral doses of ziritaxestat 600 mg q.d. on the PKs of a single dose of a representative hormonal oral contraceptive containing EE and DRSP in healthy female subjects. Results of these analyses led to further exploration of EE metabolism and the pathways that may be affected by ziritaxestat administration, including glucuronidation by UDP-glucuronosyltransferase 1A1 (UGT1A1) and sulfation by sulfotransferase family 1E member 1 (SULT1E1).

MATERIALS AND METHODS

Oral contraceptive DDI study

Study design. This was an open-label, one way crossover (fixed sequence) study enrolling healthy, nonpregnant, nonlactating women aged 18–65 years. The study was conducted at a single site in the United States between July 30, 2020, and October 21, 2020 (last subject, last scheduled visit). Subjects were screened from day −28 to day −2 and received a single dose of oral contraceptive (0.03 mg EE and 3 mg DRSP) in the fed state on days 1, 8, and 18 (**Figure 1**). There is some evidence for an impact of EE on the PKs of DRSP[4]; however, this potential interaction is not expected to impact the present study as EE and DRSP were co-administered in all three phases. From days 8 to 23, subjects received ziritaxestat 600 mg q.d. in the fed state to reduce the observed between-subject variability in ziritaxestat PK when administered in a fasted state (data on file). A fed state was defined as being 30 minutes after starting a standard breakfast (~ 545–645 kilocalories, comprising ~ 19% protein, 31% fat, 50% carbohydrates, and 4 g of fiber). Co-administration of oral contraceptive and ziritaxestat took place on 2 days to dissociate any potential inhibition from induction. On day 8, co-administration assessed the potential inhibitory effect of ziritaxestat; co-administration on day 18 occurred after potential enzyme induction to evaluate the net effect of ziritaxestat (inhibition and induction). Subjects were required to remain in the clinic throughout the study and all study medications were administered by personnel at the clinic to ensure compliance. A follow-up visit took place 14 ± 3 days after the last intake of ziritaxestat or oral contraceptive.

Ethical considerations. The study protocol, subject information sheet, and informed consent form (ICF) were reviewed and approved by an institutional review board prior to study initiation. The study was conducted in accordance with local regulations, the guidelines for Good Clinical Practice, and the principles of the current version of the Declaration of Helsinki. A signed ICF was obtained from subjects before any clinical study-related activities.

Population. Women were eligible for inclusion if they were of nonchildbearing potential or were using highly effective contraception (i.e., a nonhormonal intrauterine device, bilateral tubal occlusion, or sexual abstinence (refraining from heterosexual intercourse during the period of risk associated with the study treatments)). Active smokers or those using nicotine-containing products over the past 6 months before day 1 were excluded from the study. Study subjects were prohibited from using hormonal systemic estrogen replacement therapy and hormonal steroid-based contraceptives (oral or devices) from 7 days before the first dose of oral contraceptive on day 1 to collection of the last PK sample. All medication, including over-the-counter and/or prescription medication, dietary supplements, nutraceuticals, vitamins, and/or herbal supplements except occasional paracetamol (maximum dose 2 g/day and a maximum of 10 g/2 weeks), had to be

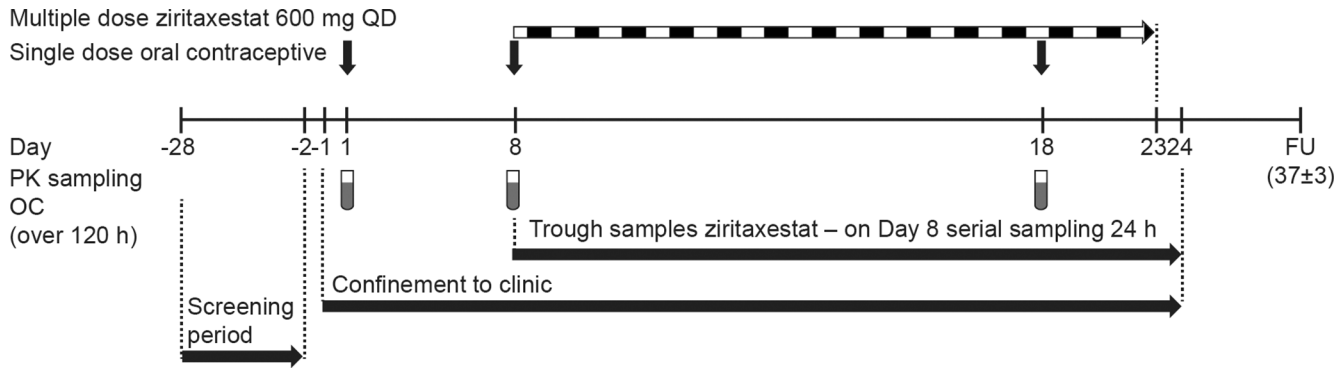


Figure 1 Oral contraceptive DDI study design. DDI, drug–drug interaction; FU, follow-up; h, hours; OC, oral contraceptive; PK, pharmacokinetic; QD, once daily.

discontinued at least 2 weeks or 5 half-lives of the drug, whichever is longer, prior to day 1 and throughout the study.

End points. The primary end point of this study was to determine the net effect of ziritaxestat on the PKs (maximum (peak) plasma drug concentration (C_{max}) and area under the plasma drug concentration–time curve from time zero to infinity (AUC_{0-inf})) of a representative hormonal oral contraceptive containing EE and DRSP.

Secondary end points aimed to determine the inhibitory effect of ziritaxestat on CYP3A4 using a hormonal oral contraceptive as a substrate and to evaluate the safety and tolerability of ziritaxestat when administered alone or in combination with an oral contraceptive. Safety parameters included treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs leading to treatment discontinuation, which were monitored throughout the study. Laboratory tests, vital signs measurement, 12-lead electrocardiograms (ECGs), and physical examinations were performed at screening, day 1, and at follow-up (full details shown in **Table S1**). Ziritaxestat PKs were also evaluated as a secondary end point in this study, but as these parameters have been described in detail previously,[3,5] they will not be reported further here. Other study end points included performing an *in vivo* analysis of SULT1E1 and UGT1A1 inhibition by ziritaxestat.

PK measurements. Oral contraceptive blood samples for PK analysis were collected on days 1, 8, and 18 (predose and then 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours postdose); days 2, 9, and 19 (24 hours postdose); and days 3–6, 10–13, and 20–23 (48, 72, 96, and 120 hours postdose). Plasma concentrations of EE and DRSP were determined by ultra-high performance liquid chromatography with tandem mass spectrometry ((UHP)LC–MS/MS) using a validated bioanalytical method (QPS, LLC). The lower limits of quantification (LLOQ) for EE and DRSP were 2.5 pg/mL and 0.5 ng/mL, respectively. PK parameters were calculated using Phoenix WinNonLin version 8.0 (Certara LP, USA) software.

In vivo analysis of SULT1E1 and UGT1A1 inhibition

Formation of EE conjugates (sulfates and glucuronides) via sulfation and glucuronidation is a major route of first-pass metabolism for EE[6]; thus, in order to measure total EE concentrations (as free and conjugated forms), EE conjugates must first be hydrolyzed in the plasma samples. Total EE concentrations were measured in plasma samples from three subjects enrolled in the oral contraceptive DDI study; these subjects were selected based on remaining plasma volumes after the quantification of ziritaxestat, DRSP, and EE. Total EE concentrations were quantified in plasma samples taken on day 1 (after administration of oral contraceptive alone) and day 18 (co-administration of oral contraceptive with ziritaxestat 600 mg q.d.) after hydrolysis of EE glucuronide and sulfate conjugates with purified genetically modified β-glucuronidase (IMCSzymes 3S) and purified arylsulfatase (Sulfazyme PaS, Development Product) provided by Integrated Micro-Chromatography Systems Inc. To differentiate the contribution of glucuronide and sulfate conjugates, each sample was analyzed in duplicate; one aliquot was treated with β-glucuronidase and the other aliquot with arylsulfatase. Chromatography was performed with water:acetic acid at 100:0.1 (v:v) (A) and methanol (B) as the mobile phase on a Waters ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column. Injection volume was 10 µL. The gradient elution scheme was: 50% B at 0–0.3 minutes, 50–70% B over 0.3–1.3 minutes, maintained to 2.3 minutes, 70–95% B from 2.3–2.4 minutes, maintained to 3.1 minutes, 95–50% B over 3.1–3.2 minutes, and 50% B from 3.2–4.3 minutes at a flow rate of ~500 µL/min. MS was performed with positive electrospray ionization in multiple reaction monitoring mode using a transition of 438.4 > 213.4 (EE) and 442.4 > 215.4 (ethinyl estradiol-d4, internal standard). Transitions were for pyridine-3-sulfonyl chloride derivatized analytes and internal standard. Control plasma samples of either ethinyl estradiol-3-glucuronide (EEG) or 17-α-ethinyl estradiol-3-sulfate (EES) were incubated with glucuronidase or arylsulfatase, respectively, before being spiked with internal standard, processed by liquid–liquid extraction, derivatized with pyridine-3-sulfonyl chloride, and analyzed by (UHP)LC-MC/MS (QPS, LLC). Hydrolysis was performed using 500 µL of plasma, 75 µL of buffer (1 M tris hydrochloride, pH 8.0), and 100 µL of enzyme; incubation was at 50°C for 1 hour, after which 50 µL of internal standard was added. Enzyme efficiency was verified by comparing total EE concentrations after hydrolysis to the EE molar equivalent of the EE conjugate concentrations.

Enzyme specificity was verified by incubating control plasma samples containing EEG with arylsulfatase and control plasma samples containing EES with glucuronidase.

SULT1E1 *in vitro* test

The inhibition potency of ziritaxestat towards the human SULT1E1 enzyme was studied using recombinant human enzymes (human SULT1E1 expressed in *Escherichia coli*) obtained from Cypex Ltd., Dundee, UK. Incubations were performed in triplicate at 37°C for 3 minutes in phosphate buffer pH 7.4 with a final dimethyl sulfoxide (DMSO) concentration of 1% and total protein concentration of 5 µg/mL. DMSO has previously been shown to have a modest impact on SULT1E1 catalytic activity and is not expected to significantly impact estimations of inhibitory activity.[7] Following a pre-incubation period of 10 minutes, the reaction was started by the addition of 3′-phosphoadenosine 5′-phosphosulfate and reactions were terminated by addition of ice-cold acetonitrile.

Ziritaxestat was used at 6 test concentrations (0.8, 2, 8, 20, 80, and 200 µM), with EE as the test substrate at a concentration of 5 nM. Quercetin at a concentration of 0.5 µM was included as positive control inhibitor, in addition to an appropriate solvent control (DMSO). The level of SULT-specific probe reaction was monitored by (UHP)LC–MS/MS and inhibition was observed as a reduced level of metabolite formation from the substrate. Chromatography was performed with aqueous 1 mM ammonium fluoride (A) and acetonitrile (B) as the mobile phase on a Waters ACQUITY UPLC High Strength Silica C18 (2.1 × 50 mm, 1.8 µm) column. Injection volume was 4 µL. The gradient elution scheme was: 5% B at 0–0.5 minutes, 5–70% B over 0.5–2 minutes, 70–95% B from 2–2.001 minutes, maintained to 3.0 minutes, 95–5% B over 3.00–3.001 minutes, and 5% B from 3.001–4 minutes at a flow rate of 0.65 mL/minute. MS was performed with negative electrospray ionization in multiple reaction monitoring mode using a transition of 375.2 > 295.2 (EE sulfate) and 351.1 > 271.1 (β-estradiol-3-sulfate, internal standard).

Statistics and calculations

Oral contraceptive DDI study statistics. Using an estimated within-subject coefficient of variation (CV)% of 25% for AUC (maximum within-subject CV% for EE and DRSP) and assuming a true geometric mean ratio (GMR) of 100%, a sample size of 12 evaluable subjects provided 90% power that the 90% confidence interval (CI) of the GMR was contained within the no effect boundaries of 80% to 125%. If a true GMR of 100% for both EE and DRSP was assumed, the power to have each individual GMR to be contained within the no effect boundaries was > 80%.

The Safety Analysis set comprised all enrolled subjects who were administered oral contraceptive or ziritaxestat at least once. The PK analysis set comprised all subjects who had available and evaluable data (excluding all protocol violations/deviations or adverse events (AEs) that could impact on the PK analysis). Descriptive statistics were

calculated by treatment day for plasma concentrations and the listed PK parameters.

DDI was evaluated on the log-transformed (natural logarithm) PK parameters (C_{max} and AUC_{0-inf} (if estimable for all subjects)) of EE and DRSP using a mixed-effects model with treatment day as the fixed effect and subject as the random effect. Point estimates of log-transformed PK parameters of EE and DRSP during intake of oral contraceptive in combination with ziritaxestat (day 8 or day 18) as test treatment vs. log-transformed PK parameters of EE and DRSP during intake of oral contraceptive alone (day 1) as reference treatment were calculated as the GMR, expressed as a percentage. To assess the potential interaction between ziritaxestat and the oral contraceptive, the 90% CI of the GMRs was calculated for the following comparisons: (i) EE and DRSP with ziritaxestat (day 18) vs. EE and DRSP alone (day 1); (ii) EE and DRSP with ziritaxestat (day 8) vs. EE and DRSP alone (day 1). There was no evidence of a DDI when the GMR and 90% CI fitted within the prespecified boundaries of 80% to 125%.

As time to maximum concentration (T_{max}) is a discrete variable dependent on selected blood sampling times, comparisons were assessed using the Wilcoxon signed rank test. No imputations were done in case of missed visits, missing values in existing records, or missing or partial date or time fields. Values for continuous parameters above/below the limit of detection were imputed with the limit of detection ± 1 unit of precision. Values for PK data below the LLOQ were imputed by 0, except for the geometric mean and the geometric CV%, where values below the LLOQ were imputed as LLOQ/2 and listed as below the LLOQ.

***In vivo* analysis of SULT1E1 and UGT1A1 inhibition – EE AUC calculation.** AUC up to the last measurable concentration (AUC_{last}) for EE with and without hydrolysis was calculated according to the linear up/logarithmic down trapezoidal method using Phoenix version 8.0 software (Certara LP, USA).

Calculation of half-maximal inhibitory concentration for SULT1E1. Half-maximal inhibitory concentration (IC_{50}) values for compounds were extrapolated by fitting the data to the following equation, where *A%* is the percentual activity remaining; *Max* is the highest fitted percentual activity; *[I]* is the inhibitor concentration; and IC_{50} is the inhibitor concentration where the remaining activity is 50%. Fitting was done using GraphPad Prism 7.00 software (GraphPad Software).

$$A\% = \frac{Max}{1 + 10^{(\mathrm{Log}[I] - \mathrm{Log}IC_{50})}}$$

Enzyme activities in the presence of inhibitors were compared with the control (incubations containing solvent but no inhibitor). Tentative inhibitory constant (K_i) values were calculated with the following equation, using measured IC_{50} values, assuming competitive inhibition and using the Cheng–Prusoff equation[8] with test substrate concentration equal to Michaelis–Menten constant K_m:

$$K_i = \frac{IC_{50}}{2}$$

RESULTS

Oral contraceptive DDI study with ziritaxestat

Population. Subject disposition is shown in **Figure S1**. Overall, 15 subjects were enrolled in the study and 13 subjects completed treatment and the study. One subject withdrew consent; a replacement subject was withdrawn from the study by the investigator due to poor venous access.

Subject demographic data are shown in **Table 1**. Median subject age was 51 years; most subjects were White. None of the study subjects had a medical history that was deemed relevant to the conduct of the study or its objective. One concurrent disease of microscopic hematuria was reported as clinically relevant at the study follow-up visit.

Primary and secondary end points. Co-administration of the oral contraceptive with ziritaxestat 600 mg q.d. resulted in a 2.8-fold increase of EE C_{max} and a 2.4-fold increase of EE AUC_{0-inf} (day 18 test vs. day 1 reference). DRSP C_{max} and AUC_{0-inf} increased by 1.1-fold and 1.2-fold, respectively (**Figure 2**, **Table 2**). Similar fold increase in EE C_{max} and AUC_{0-inf} (2.6- and 2.2-fold, respectively) and DRSP C_{max} and AUC_{0-inf} (1.1- and 1.2-fold, respectively) were noted after single co-administration of oral contraceptive with ziritaxestat (day 8 vs. day 1).

Interindividual variability in C_{max} and AUC was low and similar across the treatment days, with CV% values ranging from 16.5 to 38.6% for EE and from 15.2 to 37.0% for DRSP. The terminal half-life ($t_{1/2}$; mean (CV%)) of EE was longer on days 8 and 18 (30.1 (16.7) and 28.4 (17.0) hours, respectively) than on day 1 (22.0 (24.9) hours). EE T_{max} ranged from 2.0 to 3.0 hours with and without ziritaxestat co-administration. No major differences between the treatment days were observed for the remaining DRSP PK parameters.

In vivo analysis of SULT1E1 and UGT1A1 inhibition by ziritaxestat

Following administration of the oral contraceptive alone on day 1 or when co-administered with ziritaxestat 600 mg q.d. on day 18, the ratio of EE AUC_{last} with/without enzymatic hydrolysis by glucuronidase was roughly constant for each of the three subjects but was slightly lower on day 18 (**Figure 3**), indicating a potential weak inhibitory effect of ziritaxestat on glucuronidation.

By contrast, the ratio of EE AUC_{last} with/without enzymatic hydrolysis by arylsulfatase was substantially lower on day 18 compared to Day 1 (**Figure 3**), suggesting that ziritaxestat is a potent inhibitor of sulfation.

Table 1 Subject demographic data (safety analysis set)

Parameter	All subjects *n* = 15
Median (min, max) age, years[a]	51 (25, 65)
Baseline median (min, max) height, cm	160.5 (155.1, 172.6)
Baseline median (min, max) weight, kg	70.0 (56.7, 88.0)
Baseline median (min, max) BMI, kg/m^2	27.4 (22.4, 30.0)
Ethnicity, *n* (%)	
Hispanic/Latino	6 (40.0)
Not Hispanic/Latino	9 (60.0)
Race, *n* (%)	
Black/African American	4 (26.7)
White	11 (73.3)

BMI, body mass index.
[a]At signing of the informed consent form.

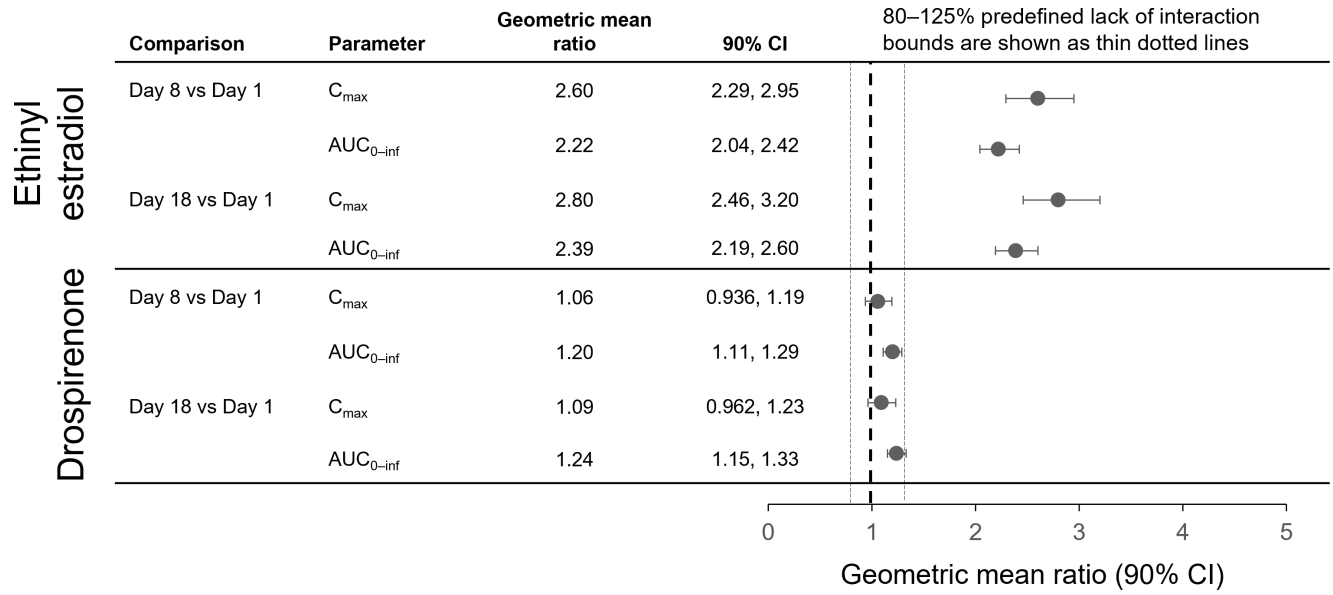


Figure 2 Forest plot of LS geometric mean ratio (90% CI) for ethinyl estradiol and drospirenone on day 18 (oral contraceptive co-administered with ziritaxestat 600 mg q.d.) vs. day 1 (oral contraceptive alone). AUC_{0-inf}, area under the plasma concentration–time curve from time zero to infinity; C_{max}, maximum (peak) plasma drug concentration; CI, confidence interval; LS, least squares.

SULT1E1 *in vitro* analysis

In vitro analysis revealed that ziritaxestat is a potent inhibitor of SULT1E1 (**Figure 4**) with an IC_{50} value of < 0.8 µM (lowest test concentration used) for the inhibition of EE. The control inhibitor quercetin incubated at 0.5 µM inhibited SULT1E1 by 66%, demonstrating the functionality of the assay (data not shown). Due to the discontinuation of the ziritaxestat program, a definitive IC_{50} value for ziritaxestat was not determined.

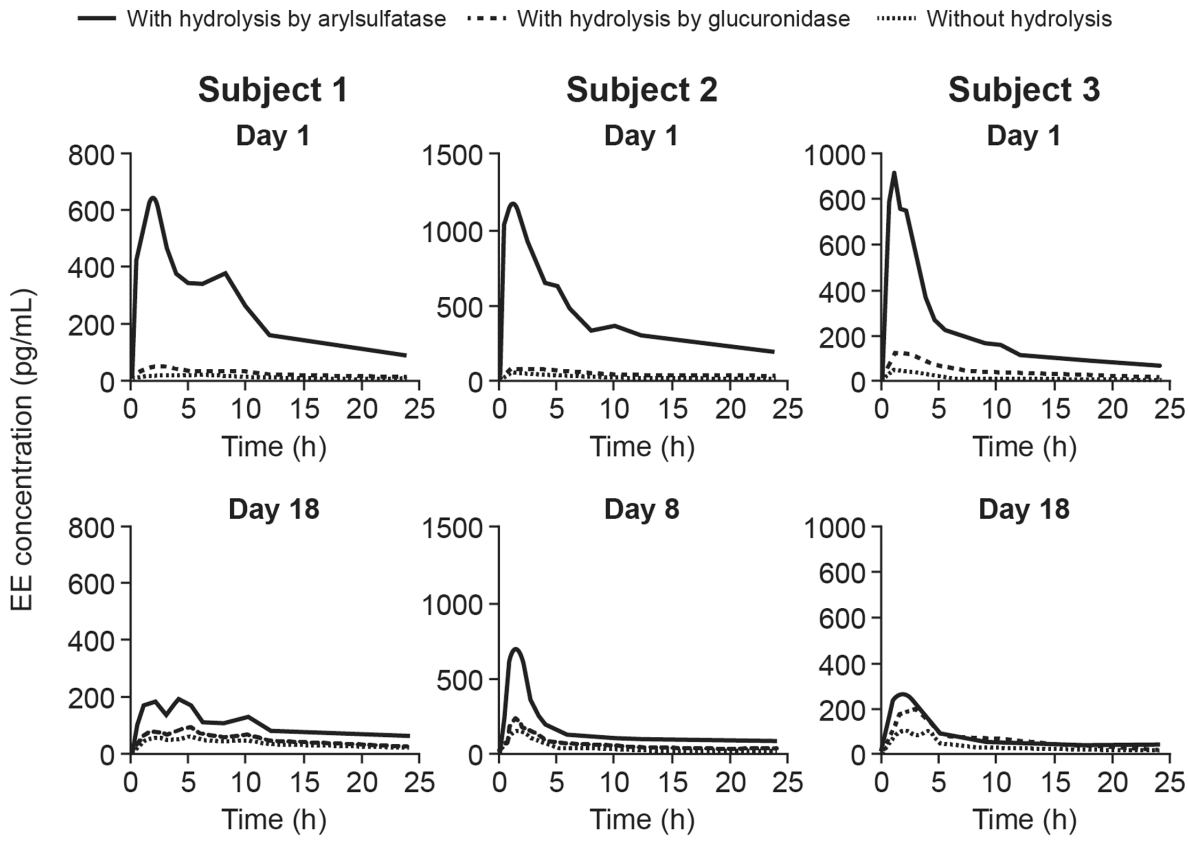
Safety summary

A summary of TEAEs is shown in **Table S2**. The proportion of subjects with ≥ 1 TEAE was higher when ziritaxestat 600 mg q.d. was co-administered with the oral contraceptive than when the oral contraceptive was administered alone (71.4 vs. 20.0%, respectively). All TEAEs were of mild intensity except one TEAE of vomiting of moderate intensity (following co-administration of oral contraceptive and ziritaxestat 600 mg q.d.). Diarrhea, nausea, and headache were the most common TEAEs on co-administration (**Table S2**) and were the most common treatment-related TEAEs with ziritaxestat. The overall incidence of TEAEs considered related to the oral contraceptive or ziritaxestat by the study investigator was the same with co-administration of these compounds (7/14 subjects (50%) for both). Reproductive system and breast disorders were reported after co-administration of oral contraceptive and ziritaxestat 600 mg q.d.; all were considered related to the oral contraceptive by the study investigator

Table 2 Descriptive statistics of selected OC PK parameters (PK analysis set)

	Mean (CV%)		
Parameter	OC (day 1), *n* = 15	OC + ziritaxestat 600 mg q.d. (day 8, SD) *n* = 14	OC + ziritaxestat 600 mg q.d. (day 18, MD) *n* = 13
Ethinyl estradiol			
AUC_{0-inf} (pg.h/mL)	967 (26.5)	2,020 (22.4)	2,180 (25.3)
C_{max} (pg/mL)	47.0 (37.3)	122 (27.6)	135 (37.3)
$t_{1/2}$ (h)	22.0 (24.9)	30.1 (16.7)	28.4 (17.0)
T_{max} (h)[a]	3.0 (1.0, 5.0)	2.0 (1.0, 5.0)	2.0 (1.0, 5.0)
Drospirenone			
AUC_{0-inf} (ng.h/mL)	574 (17.7)	686 (17.8)	708 (17.7)
C_{max} (ng/mL)	20.7 (35.8)	21.4 (28.8)	22.6 (37.0)
$t_{1/2}$ (h)	39.0 (13.9)	44.7 (8.9)	43.4 (18.3)
T_{max} (h)[a]	3.0 (1.0, 5.0)	2.5 (1.5, 5.0)	4.0 (1.5, 5.0)

AUC_{0-inf}, area under the curve from zero to infinity; C_{max}, maximum (peak) plasma drug concentration; CV, coefficient of variation; h, hours; MD, multiple dose; OC, oral contraceptive; PK, pharmacokinetic; SD, single dose; $t_{1/2}$, half-life; T_{max}, time to maximum concentration.
[a]Data are median (minimum, maximum).



	Subject 1		Subject 2		Subject 3	
	Day 1	Day 18	Day 1	Day 18	Day 1	Day 18
EE AUC_{last} ratio with/ without hydrolysis by glucuronidase	1.8	1.4	1.9	1.5	2.7	1.8
EE AUC_{last} ratio with/ without hydrolysis by arylsulfatase	16.7	2.8	16.4	3.2	11.8	2.1

Figure 3 EE plasma concentration–time profiles for three subjects following administration of oral contraceptive alone (day 1) and co-administration of oral contraceptive and ziritaxestat 600 mg once daily at steady-state (day 18), with hydrolysis by arylsulfatase or glucuronidase or without hydrolysis. AUC_{last}, area under the plasma concentration–time curve up to the last measurable concentration; EE, ethinyl estradiol; h, hours.

(one case each of breast pain, breast tenderness, and menstruation irregular, and two cases of postmenopausal hemorrhage; no cases were reported with oral contraceptive alone). No deaths or discontinuations due to AEs occurred.

Low erythrocyte counts were reported for five subjects (35.7%) on co-administration of ziritaxestat and the oral contraceptive; otherwise, there were no notable changes in clinical laboratory parameters, vital signs, or ECG parameters.

DISCUSSION

The initial oral contraceptive DDI assessment described herein showed that co-administration of an oral contraceptive with ziritaxestat 600 mg q.d. resulted in an average net (inhibition and induction effects combined) 2.8-fold and 2.4-fold increase in EE C_{max} and AUC_{0-inf}, respectively, and a 1.1-fold and 1.2-fold increase in DRSP C_{max} and AUC_{0-inf}, respectively. The similarity of the fold increase in oral contraceptive PK parameters at day 18 and day 8 with ziritaxestat co-administration suggests that inhibition occurred after a single ziritaxestat dose (day 8), and that marked induction was absent as this should have decreased the GMR at the day 18 assessment. TEAEs were numerically higher in this study with co-administration of ziritaxestat 600 mg q.d. and oral contraceptive vs. oral contraceptive alone; however, ziritaxestat was generally well tolerated with a safety profile consistent with findings from previous studies in healthy male subjects.[3,5] Ziritaxestat PK parameters following the 600 mg q.d. dose were similar to

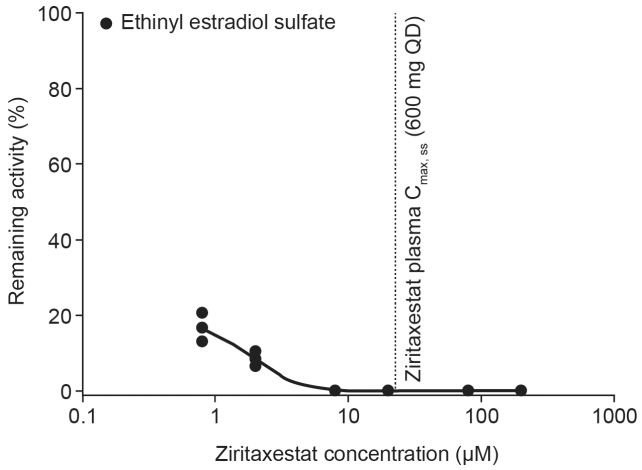


Figure 4 Observed SULT1E1 activity (n = 3, relative UHPLC–MS/MS peak areas, remaining enzyme activities) in the presence of ziritaxestat. Dotted line represents total plasma concentration of ziritaxestat 600 mg q.d. at SS. C_{max}, maximum plasma concentration; QD, once daily; SS, steady state; SULT1E1, sulfotransferase family 1E member 1; UHPLC–MS/MS; ultra-high-performance liquid chromatography with tandem mass spectrometry.

those observed in previous studies in healthy male volunteers,[3,5] indicating that subjects were exposed as expected to ziritaxestat.

The minor increase in DRSP exposure with ziritaxestat co-administration reported in the DDI study was not considered to be clinically relevant. However, the drug interaction between ziritaxestat and EE may be clinically relevant since EE doses ≥ 50 μg can increase the risk of serious adverse reactions, such as venous thromboembolic events.[2,9] Of note, the 2.4- to 2.8-fold increase in EE levels observed after co-administration with ziritaxestat would correspond with exposure to a dose > 50 μg. Importantly, DRSP is known to be a CYP3A4 substrate[1] and it is therefore unlikely that the interaction of ziritaxestat with EE is CYP3A4-related. Extensive EE metabolism occurs mainly via intestinal sulfation and hepatic oxidation, glucuronidation, and sulfation.[1] Oxidative metabolism accounts for 30% of EE dose elimination and is catalyzed by CYP3A4 and CYP2C9 (responsible for 67 and 23% of oxidative metabolism, respectively); other key EE elimination pathways include glucuronidation by UGT1A1 and sulfation by SULT1E1.[1,10] In future phase I clinical trials, monitoring of endogenous estradiol levels, alongside the non-selective UGT1A1 marker bilirubin, may help identify and differentiate potential DDIs involving inhibition of SULT1E1 and UGT1A1. The importance of identifying such biomarkers is underscored by the relatively low frequency of studies assessing potential DDIs involving phase II drug-metabolizing enzymes vs. CYP enzymes that are responsible for the bulk of drug metabolism.[11]

As a result of the oral contraceptive DDI study finding that EE exposure is increased in the presence of ziritaxestat via inhibition of an enzyme other than CYP3A4, an *in vivo* analysis of SULT1E1 and UGT1A1 inhibition by ziritaxestat was performed and the potency of *in vitro* inhibition of SULT1E1 by ziritaxestat was assessed. *In vivo* assessments using plasma samples from the DDI study revealed that glucuronidation of EE was weakly inhibited by ziritaxestat. The more notable finding was that ziritaxestat strongly inhibited sulfation of EE and that this mechanism was likely responsible for the clinically relevant increases in EE exposure in the DDI study. *In vitro* assessments of this potential interaction confirmed that ziritaxestat is a potent inhibitor of SULT1E1.

SULT1E1 is a key enzyme in estrogen homeostasis expressed in hormone-dependent tissues, such as the endometrium.[12] The potential for interactions between EE and SULT1E1 is reported in the FDA guidance regarding the assessment of DDIs with oral contraceptives[2]; our results represent the first report of the effects of a potent inhibitor of SULT1E1 on EE clearance in a clinical trial setting. Although the present analyses were initiated as part of a standard oral contraceptive–investigational compound DDI study, taken as a whole, the findings from this study and the resultant additional enzyme assessments may have broader relevance for investigating potential oral contraceptive DDIs in the future. Our results clearly indicate that DDIs with enzymes other than CYP3A4 can result in clinically relevant changes in exposure to oral contraceptives. We assert that assessments of additional metabolic pathways should be considered as part of the standard evaluation of oral contraceptive DDIs and we describe herein the methodology of a SULT1E1 inhibition assay that could be incorporated into comprehensive DDI evaluations in the future.

Limitations of these analyses include the absence of a clear estimate of the IC_{50} of ziritaxestat for SULT1E1. These additional data were not viewed as scientifically valuable due to the discontinuation of the ziritaxestat program, although the authors appreciate that they may have added to the completeness of the presented narrative. The small number of subjects studied *in vivo* (n = 3), and the absence of an *in vitro* assessment of UGT1A1 inhibition by ziritaxestat could also be viewed as limitations. UGT1A1 transforms small lipophilic molecules, such as steroid hormones and bilirubin, into water-soluble, excretable metabolites.[13] As no issues with total or conjugated bilirubin were flagged as part of the laboratory parameter safety panels in phase I randomized trials of ziritaxestat up to doses of 1,000 mg q.d.,[5] this was taken as indirect confirmation that ziritaxestat is not an UGT1A1 inhibitor, and no further assessments regarding UGT1A1 were performed.

In conclusion, multiple dosing with ziritaxestat 600 mg q.d. resulted in a 2.4- to 2.8-fold increase in EE exposure while no clinically relevant increase was observed for DRSP exposure. DRSP is a sensitive CYP3A4 substrate, and therefore interactions between ziritaxestat and alternative oral contraceptive metabolic pathways were analyzed to explain the increase in EE exposure. *In vivo* and *in vitro* investigations revealed that EE exposure increased with co-administration of an oral contraceptive and ziritaxestat because ziritaxestat is a potent inhibitor of sulfation via SULT1E1. To our knowledge, this is the first time the effect of a potent inhibitor of SULT1E1 on EE clearance has been reported in a clinical setting; this finding indicates the necessity to extend DDI investigations to include assessment of UGT and SULT enzymes.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

F.S.N. and S.L.T. are employees of Galapagos SASU. K.V.A. is an employee of Galapagos NV. E.H., I.S. and N.K. are former employees of Galapagos NV. G.S. is a consultant for Galapagos SASU. G.C. is an employee of Gilead Sciences, Inc.

AUTHOR CONTRIBUTIONS

F.S.N., K.V.A., N.K., E.H., I.S., S.L.T., G.C., and G.S. wrote the manuscript and designed the research. K.V.A., N.K., E.H., and I.S. performed the research. F.S.N., N.K., E.H., I.S., S.L.T., G.C., and G.S. analyzed the data.

1. Zhang, N. *et al*. Role of CYP3A in oral contraceptives clearance. *Clin. Transl. Sci.* **11**, 251–260 (2018).
2. US Food and Drug Administration. Clinical drug interaction studies with combined oral contraceptives guidance for industry <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-drug-interaction-studies-combined-oral-contraceptives-guidance-industry> (2020).
3. Helmer, E. *et al*. Pharmacokinetics and metabolism of ziritaxestat (GLPG1690) in healthy male volunteers following intravenous and oral administration. *Clin. Pharmacol. Drug Dev.* **11**, 246–256 (2022).
4. Richter, W.H., Koytchev, R., Kirkov, V., Merki, G., Colli, E. & Regidor, P.A. Comparative pharmacokinetic estimates of drospirenone alone and in combination with ethinyl estradiol after single and repeated oral administration in healthy females. *Contraception* **101**, 137–143 (2020).
5. van der Aar, E. *et al*. Safety, pharmacokinetics, and pharmacodynamics of the autotaxin inhibitor GLPG1690 in healthy subjects: phase 1 randomized trials. *J Clin. Pharmacol.* **59**, 1366–1378 (2019).
6. Raftogianis, R., Creveling, C., Weinshilboum, R. & Weisz, J. Estrogen metabolism by conjugation. *J. Natl. Cancer Inst. Monogr.* **2000**, 113–124 (2000).
7. Ma, B., Shou, M. & Schrag, M.L. Solvent effect on cDNA-expressed human sulfotransferase (SULT) activities in vitro. *Drug Metab. Dispos.* **31**, 1300–1305 (2003).
8. Cheng, Y. & Prusoff, W.H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **22**, 3099–3108 (1973).
9. Gerstman, B.B., Piper, J.M., Tomita, D.K., Ferguson, W.J., Stadel, B.V. & Lundin, F.E. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. *Am. J. Epidemiol.* **133**, 32–37 (1991).
10. Zhang, H. *et al*. Pharmacokinetic drug interactions involving 17alpha-ethinylestradiol: a new look at an old drug. *Clin. Pharmacokinet.* **46**, 133–157 (2007).
11. Cerny, M.A. Prevalence of non-cytochrome P450-mediated metabolism in Food and Drug Administration-approved oral and intravenous drugs: 2006–2015. *Drug Metab. Dispos.* **44**, 1246–1252 (2016).
12. Yi, M., Negishi, M. & Lee, S.J. Estrogen sulfotransferase (SULT1E1): its molecular regulation, polymorphisms, and clinical perspectives. *J. Pers. Med.* **11**, 194 (2021).
13. Levesque, E., Girard, H., Journault, K., Lepine, J. & Guillemette, C. Regulation of the UGT1A1 bilirubin-conjugating pathway: role of a new splicing event at the UGT1A locus. *Hepatology* **45**, 128–138 (2007).