Effects of Strong Inhibition of CYP3A and UGT1A9 and Strong Induction of CYP3A on the Pharmacokinetics, Safety, and Tolerability of Soticlestat: Two Drug–Drug Interaction Studies in Healthy Volunteers

Wei Yin, Cheng Dong, Annette Stevenson, Valerie Lloyd, Marco Petrillo,* Mike Baratta, Tom Hui, and Steve Han


*At the time the study was conducted.

Running title: Soticlestat Drug–Drug Interactions in Healthy Adults

Corresponding author: Wei Yin, PhD, Takeda Development Center Americas, Inc., 35 Landsdowne St, Cambridge, MA 02139, USA. Email: wei.yin@takeda.com; Telephone: 617-679-7000; fax: 617-551-8650; ORCID ID: orcid.org/0000-0002-4834-5783
Nonstandard abbreviations: AE, adverse event; AUC$_{\infty}$, area under the concentration–time curve from time 0 to infinity; AUC$_{\text{extrap}}$, area under the concentration–time curve from last quantifiable concentration to infinity expressed as a percentage; AUC$_{\text{last}}$, area under the concentration–time curve from time 0 to time of last quantifiable concentration; BMI, body mass index; C-SSRS, Columbia Suicide Severity Rating Scale; CH24H, cholesterol 24-hydroxylase; CI, confidence interval; CL/F, apparent clearance after extravascular administration; C$_{\text{max}}$, maximum observed concentration; CYP, cytochrome P450; DDI, drug–drug interaction; GMR, geometric mean ratio; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LSM, least-squares mean; n/a, not applicable; PK, pharmacokinetics; $t_{1/2z}$, terminal disposition phase half-life; TEAE, treatment-emergent adverse event; $t_{\text{max}}$, time of first occurrence of maximum observed concentration; UGT, UDP glucuronosyltransferase; Vz/F, apparent volume of distribution during the terminal disposition phase after extravascular administration.
Abstract

Two open-label, phase 1 studies (NCT05064449, NCT05098041) investigated the effects of CYP3A inhibition (via itraconazole), UGT1A9 inhibition (via mefenamic acid), and CYP3A induction (via rifampin) on the pharmacokinetics of soticlestat and its metabolites M-I and M3. In period 1 of both studies, participants received a single dose of soticlestat 300 mg. In period 2, participants received itraconazole on days 1–11 and soticlestat 300 mg on day 5 (itraconazole/mefenamic acid study; part 1); mefenamic acid on days 1–7 and soticlestat 300 mg on day 2 (itraconazole/mefenamic acid study; part 2); or rifampin on days 1–13 and soticlestat 300 mg on day 11 (rifampin study). Twenty-eight healthy adults participated in the itraconazole/mefenamic acid study (14 per part) and 15 participated in the rifampin study (mean age, 38.1–40.7 years; male, 79–93%). For maximum observed concentration, the geometric mean ratios (GMRs) of soticlestat + itraconazole, mefenamic acid, or rifampin to soticlestat alone were 116.6%, 107.3% and 13.2%, respectively, for soticlestat; 10.7%, 118.0% and 266.1%, respectively, for M-I, and 104.6%, 88.2% and 66.6%, respectively, for M3. For area under the curve from time 0 to infinity, the corresponding GMRs were 124.0%, 100.6%, and 16.4% for soticlestat; 13.3%, 117.0%, and 180.8% for M-I; and 120.3%, 92.6%, and 58.4% for M3. Soticlestat can be administered with strong CYP3A and UGT1A9 inhibitors, but not strong CYP3A inducers (except for antiseizure medications, which will be further evaluated in ongoing phase 3 studies). In both studies, all treatment-emergent adverse events were mild or moderate.
Significance Statement

These drug–drug interaction studies improve our understanding of the potential changes that may arise in soticlestat exposure in patients being treated with CYP3A inhibitors, UGT1A9 inhibitors, or CYP3A inducers. The results build on findings from previously published soticlestat studies and provide important information to help guide clinical practice. Soticlestat has shown positive phase 2 results and is currently in phase 3 development for the treatment of seizures in patients with Dravet syndrome and Lennox–Gastaut syndrome.
Introduction

Dravet syndrome and Lennox–Gastaut syndrome are rare developmental and epileptic encephalopathies that are characterized by frequent seizures and developmental disability (Scheffer et al., 2017; Asadi-Pooya, 2018). Treatments for patients with Dravet syndrome or Lennox–Gastaut syndrome focus on reducing the frequency of the most incapacitating seizures (Asadi-Pooya, 2018; Strzelczyk and Schubert-Bast, 2022); however, resistance to antiseizure medications is common, and therapy with multiple antiseizure medications and changes to treatment over time are often needed to address patients' individual needs. The concurrent use of multiple antiseizure medications can increase the risk of drug–drug interactions (DDIs) and potential adverse events (AEs), such as increased seizure frequency, and can deter adherence to treatment (de Leon, 2015; Verrotti et al., 2020). As such, new therapies are needed to improve tolerability and to decrease the risk of DDIs in patients with Dravet syndrome or Lennox–Gastaut syndrome (Resnick and Sheth, 2017; Strzelczyk and Schubert-Bast, 2022).

Soticlestat is a first-in-class inhibitor of cholesterol 24-hydroxylase (CH24H, also known as the cytochrome P450 [CYP] enzyme CYP46A1) (Koike et al., 2022) that is in phase 3 development for the treatment of seizures in patients with Dravet syndrome or Lennox–Gastaut syndrome (ClinicalTrials.gov: NCT04940624; NCT04938427; NCT05163314). The pharmacokinetic (PK) properties of soticlestat have been characterized in various studies. Findings have shown that renal excretion of soticlestat is limited; less than 1% of administered soticlestat is recovered in urine and the mean renal clearance value for soticlestat is estimated to be 0.2 (standard deviation [S.D.], 0.11) L/h. The majority of soticlestat is rapidly metabolized by glucurondiation via UDP glucuronosyltransferases (UGTs) 2B4 (fraction metabolized: 81.2%) and 1A9 (fraction metabolized: 9.3%) to the glucuronide M3, and by oxidation via CYP3A4/5 to the aromatic N-oxide M-I (fraction metabolized: 9.5%), with M-I or M3 demonstrating negligible biological activity compared with soticlestat (Yin et al., 2023). Plasma protein binding of
soticlestat was found to be concentration dependent, ranging from 70.6% at 10 μg/mL to 94.0% at 0.1 μg/mL in vitro, while there is limited partitioning of soticlestat into blood cells, as demonstrated by mean whole blood:plasma partitioning ratios of 0.5–0.7 (Yin et al., 2023). Furthermore, soticlestat is highly soluble and highly permeable compared to a reference high permeability compound ([³H]propranolol). The clinical dose of 300 mg is soluble in 250 mL of aqueous media over the pH range of 1.2 to 6.8, while the permeability of soticlestat in Caco-2 cells from the apical to the basal side is $15.5 \times 10^{-6}$ cm/sec.

In humans, the majority of soticlestat is rapidly metabolized by glucuronidation via UDP glucuronosyltransferases (UGTs) 2B4 (fraction metabolized, 81.2%) and 1A9 (fraction metabolized, 9.3%) to the glucuronide M3, and by oxidation via CYP3A4/5 to the aromatic N-oxide M-I (fraction metabolized, 9.5%). Metabolites M-I and M3 demonstrate negligible biological activity compared with soticlestat (Yin et al., 2023). Many medications are inhibitors or inducers of CYP3A, UGT1A9, or UGT2B4, including commonly prescribed antiseizure medications, and could affect the plasma exposure of soticlestat (de Leon, 2015). Consequently, identifying potential DDIs is important to ensure that soticlestat exposure remains within an acceptable range for efficacy and safety when administered with concomitant medications.

In phase 1 and 2 studies, soticlestat was well tolerated in healthy adults after single doses of up to 1350 mg and in healthy adults and patients with developmental and epileptic encephalopathies (including Dravet syndrome or Lennox–Gastaut syndrome) after multiple doses of up to 300 mg twice daily (Halford et al., 2021; Wang et al., 2021; Hahn et al., 2022; Wang et al., 2022). To determine the extent of any potential changes to soticlestat exposure and to inform dose adjustment recommendations, we investigated and characterized the effects of strong CYP3A inhibition via itraconazole, strong CYP3A induction via rifampin, and strong UGT1A9 inhibition via mefenamic acid on the PK of soticlestat in healthy adults.
Materials and Methods

Ethics

Both studies were conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice (E6) and the Declaration of Helsinki. All other ethics guidelines, applicable laws and local regulations were followed. All study documents were approved by the Institutional Review Board or the Research Ethics Committee of the study site(s) before study initiation. All participants provided written informed consent.

Itraconazole and Mefenamic Acid Study

Study Design and Dose Selection

This open-label, two-part, phase 1 study was conducted at a single site in the USA between October 2021 and November 2021 (ClinicalTrials.gov identifier: NCT05064449). Each part of the study was conducted as a two-period, fixed-sequence design (Supplementary Fig. 1A) and participants who enrolled in part 1 were independent from participants who enrolled in part 2. Dose selection for soticlestat was based on the maximum twice-daily dose (300 mg) that is under investigation in phase 3 studies (NCT04940624; NCT04938427; NCT05163314). Dose selection for itraconazole and mefenamic acid was based on clinical recommendations (FDA; Chen et al., 2019).

In part 1, soticlestat (Bushu Pharmaceuticals Ltd., Saitama, Japan) was administered as a single 300 mg dose orally (three 100 mg tablets) on day 1 after an overnight fast of at least 10 hours, followed by a washout period of 4 days (period 1). Itraconazole 200 mg (Janssen Pharmaceutica NV, Beerse, Belgium) was then administered once daily as a solution on days 1–4 to achieve maximum CYP3A inhibition before soticlestat administration (Chen et al., 2019). On the morning of day 5, after an overnight fast of at least 10 hours, soticlestat was administered as a single 300 mg dose orally with itraconazole 200 mg. Itraconazole 200 mg was administered alone on days 6–11 to maintain CYP3A inhibition (period 2).
In part 2, soticlestat was administered as a single 300 mg dose orally (three 100 mg tablets) on day 1 after an overnight fast of at least 10 hours, followed by a washout period of 4 days (period 1). Mefenamic acid (Micro Labs Limited, Bangalore, India) was administered every 6 hours alone on day 1, with an initial dose of 500 mg in the morning; all subsequent doses were 250 mg. On the morning of day 2, mefenamic acid 250 mg was administered with soticlestat 300 mg after a fasting period of at least 6 hours, followed by mefenamic acid 250 mg alone on days 3–7 to maintain UGT1A9 inhibition (period 2).

In periods 1 and 2 of parts 1 and 2, blood samples for PK evaluation of soticlestat and its metabolites were collected before dose administration, and at 0.133, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 14, 24, 36, 48, 72, and 96 hours after dose administration. In period 2 of part 1, blood samples were collected at 120, 144, and 168 hours after soticlestat dose administration. In period 2 of part 2, blood samples were collected at 120 and 144 hours after soticlestat dose administration. Plasma concentrations of soticlestat, M-I, and M3 were determined by a validated sensitive and specific liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based method (API-5500 mass spectrometer; SCIEX, Framingham, MA, USA). The bioanalytical methods of soticlestat, M-I and M3 are described in the Supplementary Materials. A safety follow-up phone call was conducted within 13–17 days of the last soticlestat dose.

Study Participants

Eligible participants were healthy, male or female of non-childbearing potential, non-smoking adults aged 19–55 years, who had a body mass index (BMI) of 18.0–32.0 kg/m². Key exclusion criteria included: any past or present clinically significant epilepsy, seizure, or convolution, or tremor or related symptoms; any pre-existing conditions that may affect the absorption, distribution, metabolism, or elimination of the study drug; known or suspected poor CYP2C9 metabolizers based on previous history or experience with other CYP2C9 substrates (part 2 only, because mefenamic acid undergoes glucuronidation and is also metabolized by CYP2C9 to 3-hydroxymethyl mefenamic acid).
Study Objectives and Endpoints

The primary objective was to assess the effect of multiple doses of itraconazole and mefenamic acid on the single-dose PK of soticlestat. The secondary objective was to evaluate safety and tolerability of soticlestat following a single oral dose of soticlestat in the presence or absence of itraconazole, a strong CYP3A inhibitor, and mefenamic acid, a strong UGT1A9 inhibitor. The primary endpoints were maximum observed concentration (C\text{max}), area under the concentration–time curve from time 0 to infinity (AUC\text{∞}), and area under the concentration–time curve from time 0 to time of last quantifiable concentration (AUC\text{last}), and time of first occurrence of \( C_{\text{max}} \) (\( t_{\text{max}} \)) for soticlestat alone compared with soticlestat administered with itraconazole or mefenamic acid. Exploratory endpoints included the PK parameters for metabolites. Safety assessments included the incidence of AEs, evaluation using the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2011), electrocardiogram (ECG) assessments, vital signs, and laboratory tests. Treatment-emergent adverse events (TEAEs) were defined as AEs that were starting or worsening at the time of, or after, study drug administration. Each TEAE was attributed to a treatment, based on the onset date and time of the AE.

Data Analysis

The PK analysis population included all participants who adhered to the study protocol and had interpretable PK data. The safety analysis population included participants who received at least one dose of soticlestat or soticlestat + itraconazole or mefenamic acid. Fourteen participants were enrolled in part 1 and a further 14 participants were enrolled in part 2 to ensure that 12 participants completed each part of the study.

The effect of coadministration of itraconazole or mefenamic acid on key PK parameters (AUC\text{∞}, AUC\text{last}, C\text{max}) of soticlestat, M-I, and M3 was assessed by a linear mixed-effects model. The geometric mean ratios (GMRs) for the log-transformed PK parameters of soticlestat + itraconazole or mefenamic acid/soticlestat alone and associated 90% confidence intervals (CIs) were calculated with treatment as a fixed effect and participant as a random effect. The use of
90% CIs was based on clinical guidance (FDA, 2020). PK analyses were summarized descriptively by treatment and PK parameters by sample size (n); arithmetic mean and coefficient of variation (CV in per cent [CV%]); geometric mean and CV%; S.D.; standard error of the mean; minimum value; median value; and maximum value. A non-parametric analysis for paired samples (Wilcoxon signed-rank test) was used to analyze the $t_{\text{max}}$ data for plasma soticlestat, M-I, and M3. The difference of medians (treatment effect) for soticlestat + itraconazole or mefenamic acid/soticlestat alone and the corresponding 90% CIs were estimated using the Hodges–Lehmann method and Walsh averages. The $t_{\text{max}}$ values were not log-transformed.

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc, Cary, NC, USA). PK analyses were performed using Phoenix WinNonlin software, version 8.3 (Certara, Princeton, NJ, USA).

**Rifampin Study**

*Study Design and Dose Selection*

This open-label, phase 1 study was conducted at a single site in Northern Ireland, UK, between November 2021 and March 2022 (ClinicalTrials.gov identifier: NCT05098041). The study was conducted as a two-period, fixed-sequence design (Supplementary Fig. 1B). Dose selection for soticlestat was based on the maximum twice-daily dose (300 mg) that is under investigation in phase 3 studies (NCT04940624; NCT04938427; NCT05163314). Dose selection for rifampin was based on clinical recommendations and similar DDI studies (Tortorici et al., 2014).

In period 1, soticlestat was administered as a single 300 mg dose orally (three 100 mg tablets) on day 1 under fasting conditions, followed by a washout period of 4 days. In period 2, rifampin 600 mg (two 300 mg capsules; Sanofi, Berkshire, UK) was administered once daily on days 1–13 under fasting conditions to achieve maximum CYP3A induction (Chen et al., 2019). On the morning of day 11, soticlestat was administered as a single 300 mg dose orally with the rifampin 600 mg dose.
PK blood samples for soticlestat and its metabolites were collected before dose administration in periods 1 and 2, and at 0.133, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 14, 24, 36, 48, 72, and 96 (period 1 only) hours after dose administration. The sample at 96 hours corresponded to the pre-dose sample for period 2. Plasma concentrations of soticlestat, M-I, and M3 were determined by a validated sensitive and specific LC-MS/MS-based method (full methods described in the Supplementary Materials). A safety follow-up phone call was conducted within 13–17 days of the last soticlestat dose.

Study Participants

Eligible participants were healthy, non-smoking adults aged 18–55 years who had a BMI of 18.0–32.0 kg/m². Key exclusion criteria included: any past or present clinically significant epilepsy, seizure, or convulsion, or tremor or related symptoms; any pre-existing conditions that may affect the absorption, distribution, metabolism, or elimination of the study drug; and females of childbearing potential.

Study Objectives and Endpoints

The primary objective was to assess the effect of multiple doses of rifampin on the single-dose PK of soticlestat. The secondary objective was to evaluate safety and tolerability of soticlestat following a single oral dose in the presence or absence of rifampin, a strong CYP3A inducer. The primary endpoints were $C_{\text{max}}$, $AUC_{\infty}$, $AUC_{\text{last}}$, and $t_{\text{max}}$ for soticlestat alone compared with soticlestat + rifampin. Exploratory endpoints included the PK parameters for the metabolites M-I and M3. Safety assessments included the incidence of TEAEs among participants, ECG assessments, vital signs, laboratory evaluations, and C-SSRS.

Data Analysis

The PK analysis population included all participants who adhered to the study protocol and had interpretable PK data. The safety analysis population included participants who received at least one dose of soticlestat or rifampin. In total, 14 participants were enrolled to
ensure that at least 12 participants completed the study. A 15th participant was enrolled as a replacement for one participant who prematurely discontinued the study.

The effects of the coadministration of rifampin on key PK parameters (AUC\textsubscript{∞}, AUC\textsubscript{last}, C\textsubscript{max}) of soticlestat, M-I, and M3 were assessed by a linear mixed-effects model. The GMRs for the log-transformed PK parameters of soticlestat + rifampin/soticlestat alone and associated 90% CIs were calculated with treatment as a fixed effect and participant as a random effect. PK analyses were summarized descriptively by treatment and PK parameters by sample size (n); arithmetic mean and CV%; geometric mean and CV%; S.D.; standard error of the mean; minimum value; median value; and maximum value. A non-parametric analysis for paired samples (Wilcoxon signed-rank test) was used to analyze the t\textsubscript{max} data for plasma soticlestat, M-I, and M3. The difference of medians (treatment effect) for soticlestat + itraconazole or MA/soticlestat alone and the corresponding 90% CI were estimated using the Hodges–Lehmann method and Walsh averages. The t\textsubscript{max} values were not log-transformed.

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc, Cary, NC, USA). PK analyses were performed using Phoenix WinNonlin software, version 8.3 (Certara, Princeton, NJ, USA).

Results

Itraconazole and Mefenamic Acid Study

Baseline Demographics and Characteristics of Participants

All of the 28 enrolled healthy volunteers (14 per part) completed the study. For part 1, most participants were male (78.6%) and White (57.1%) and, for part 2, most were male (85.7%) and White (35.7%) or Black/African American (35.7%) (Table 1). The mean (S.D.) age was 40.7 (9.2) years for participants in part 1, with a mean (S.D.) weight of 80.2 (11.9) kg and a mean (S.D.) BMI of 26.3 (2.7) kg/m\textsuperscript{2}. For participants in part 2, the mean (S.D.) age was 40.2
(8.3) years, the mean (S.D.) weight was 84.9 (12.2) kg, and the mean (S.D.) BMI was 28.9 (3.0) kg/m².

**PK Analysis**

Key PK parameters for soticlestat, M-I, and M3 (soticlestat ± itraconazole or MA) are presented in Table 2, with PK analysis summarized in Table 3. The GMRs for Cₘₐₓ of soticlestat with itraconazole or mefenamic acid compared with soticlestat alone were 116.6% and 107.3%, respectively, for soticlestat; 10.7% and 118.0%, respectively, for M-I; and 104.6% and 88.2%, respectively, for M3. For AUCᵢₙ, the corresponding GMRs were 124.0% and 100.6%, respectively, for soticlestat; 13.3% and 117.0%, respectively, for M-I; and 120.3% and 92.6%, respectively, for M3. No statistical differences were observed in the tₘₐₓ values between soticlestat with itraconazole or mefenamic acid and soticlestat alone (all P > 0.05). The mean soticlestat plasma concentration–time curves for both parts of the study were similar in the presence or absence of itraconazole or mefenamic acid, and are presented in Fig. 1A and Fig. 1B, respectively (individual plots are provided in Supplementary Fig. 2 and 3).

**Safety**

In part 1, eight TEAEs were experienced by three participants (21.4%) (Table 4). The most frequently reported TEAE was nausea (n = 2, 14.3%). Seven TEAEs were mild in severity, and one was moderate (headache following itraconazole alone). The investigator considered three TEAEs to be related to itraconazole alone, three TEAEs to be related to soticlestat and itraconazole, and two TEAEs to be unrelated to soticlestat or itraconazole.

In part 2, 11 TEAEs were experienced by four participants (28.6%). None of the reported TEAEs occurred in more than one participant and all were mild in severity. The investigator considered four TEAEs to be related to soticlestat alone, two TEAEs to be related to mefenamic acid alone, and five TEAEs to be related to soticlestat and mefenamic acid.
There were no serious TEAEs, severe TEAEs, TEAEs leading to treatment discontinuation, or deaths. No trends were noted in physical examinations, vital signs, laboratory tests, C-SSRS results, or ECG data for participants during the study. No participants had an interruption or reduction in dosages during the study.

Rifampin Study

Baseline Demographics and Characteristics of Participants

Of the 15 healthy volunteers who were enrolled in the study and received the study drug(s), 14 completed the study. One participant discontinued the study early after withdrawing their consent to participate on day 1 of period 1 owing to personal reasons and another participant was enrolled as a replacement. Most participants were male (93%) and all were White (Table 1). The mean (S.D.) age of participants was 38.1 (10.1) years, with a mean (S.D.) weight of 80.7 (10.2) kg and a mean (S.D.) BMI of 26.7 (3.2) kg/m$^2$.

PK Analysis

Key PK parameters for soticlestat, M-I, and M3 (soticlestat ± rifampin) are summarized in Table 5, with PK analysis presented in Table 6. The GMR of soticlestat with rifampin compared with soticlestat alone for $C_{\text{max}}$ was 13.2% for soticlestat, 266.1% for M-I, and 66.6% for M3. For AUC$_{\infty}$, the corresponding GMR was 16.4% for soticlestat, 180.8% for M-I, and 58.4% for M3. No statistical difference was observed in the $t_{\text{max}}$ value between soticlestat with rifampin compared with soticlestat alone ($P > 0.05$). The mean soticlestat plasma concentration–time curve demonstrates that soticlestat exposure is reduced in the presence of rifampin compared with soticlestat alone (Fig. 1; individual plots are provided in Supplementary Fig. 4).
Safety

Nine TEAEs were experienced by four participants (26.7%) (Table 4). None of the reported TEAEs occurred in more than one participant and all were mild or moderate in severity. The investigator considered two TEAEs to be related to rifampin, and all other TEAEs to be unrelated to soticlestat or itraconazole. There were no serious TEAEs, severe TEAEs, TEAEs leading to treatment discontinuation, or deaths. No trends were noted in physical examinations, vital signs, laboratory tests, C-SSRS results, or ECG data for participants during the study. No participants had an interruption or reduction in dosages during the study.

Discussion

Based on the negligible effects of itraconazole or mefenamic acid on soticlestat PK, the potential for a clinically important DDI between soticlestat and CYP3A or UGT1A9 inhibitors is low. However, strong CYP3A inducers are not recommended to be administered with soticlestat, given the substantial and potentially clinically significant reduction in overall and peak exposure of soticlestat when administered with rifampin (AUC$_\infty$, –84%; AUC$_{\text{last}}$, –85%; C$_{\text{max}}$, –87%). Antiseizure medications that are CYP3A inducers (such as carbamazepine, phenobarbital, phenytoin, oxcarbazepine, and clobazam) (de Leon, 2015) constitute an exception because they have not been found to affect the total plasma exposure of soticlestat in phase 2 studies. Administration of soticlestat with antiseizure medications that are CYP3A inducers will be further monitored in ongoing phase 3 studies, and population PK analyses will be performed to evaluate the impact of these antiseizure medications on soticlestat exposure upon study completion. Given the polytherapy approach to treating patients with Dravet syndrome or Lennox–Gastaut syndrome, dose adjustments will be specific to individuals if a DDI should occur. The findings from the present studies were used to inform protocol development for the subsequent phase 2 and phase 3 studies.
Findings from these two DDI studies support CYP3A as an important metabolic pathway for soticlestat (Yin et al., 2023). CYP3A inhibition by itraconazole had minimal effects on the PK of soticlestat and the glucuronide of soticlestat, M3, but reduced the levels of the aromatic N-oxide of soticlestat, M-I. Conversely, CYP3A induction by rifampin substantially affected the PK of soticlestat, resulting in a large decrease in AUC and $C_{\text{max}}$ for soticlestat that was accompanied by a substantial increase in M-I levels and a moderate decrease in M3 levels. Although UGT1A9 inhibition by mefenamic acid had little to no effect on the PK of soticlestat, a slight increase in M-I was observed alongside a comparable decrease in M3. Overall, the increase in M-I levels following CYP3A induction indicates that metabolism of soticlestat via CYP3A can become dominant, despite glucuronidation to M3 representing the major metabolic pathway for soticlestat (Yin et al., 2023).

After a 7-day course of itraconazole and a single 300 mg dose of soticlestat, the overall exposure of soticlestat was minimally increased ($AUC_{\infty}$, 24% and $AUC_{\text{last}}$, 22%) in the presence of itraconazole, while $C_{\text{max}}$ was only 17% higher with soticlestat + itraconazole. Although PK parameters for M3 were not increased considerably, the exposure of M-I was greatly reduced by itraconazole coadministration. However, given that M-I is not considered an active metabolite, and changes to soticlestat exposure were minimal, none of the effects of itraconazole were considered clinically meaningful. Therefore, the risk of a DDI when soticlestat is coadministered with CYP3A inhibitors is very low.

In part 2 of the itraconazole and mefenamic acid study, following a 7-day course of mefenamic acid and a single 300 mg dose of soticlestat, the overall and peak exposures of soticlestat were very minimally increased ($AUC_{\infty}$, 1%; $AUC_{\text{last}}$, 7%; $C_{\text{max}}$, 7%) and M-I exposure also showed minimal increases ($AUC_{\infty}$, 17%; $AUC_{\text{last}}$, 20%; $C_{\text{max}}$, 18%). M3 exposure was minimally reduced ($AUC_{\infty}$, -7%; $AUC_{\text{last}}$, -8%; $C_{\text{max}}$, -12%) after coadministration of the UGT1A9 inhibitor mefenamic acid. Given that glucuronidation was found to be the major metabolic pathway for soticlestat in a mass balance study, these results suggest that soticlestat can be
metabolized to M3 by other UGTs (such as UGT2B4) in the absence of UGT1A9 (Yin et al., 2023). Similarly to M-I, M3 is not considered an active metabolite.

The effects of mefenamic acid on the PK of soticlestat or its main metabolites were not considered to be clinically meaningful, given that soticlestat exposure was hardly affected by UGT1A9 inhibition. Therefore, the risk of a DDI when soticlestat is coadministered with UGT1A9 inhibitors is very low. Many commonly prescribed antiseizure medications demonstrate inhibitory activity towards UGTs, including valproate, lamotrigine, and clobazam (de Leon, 2015). These results provide reassurance that soticlestat exposure is likely to be unchanged and remain in a therapeutic range when coadministered with UGT1A9 inhibitors. Although soticlestat is primarily metabolized by UGT2B4, currently there is no specific and potent UGT2B4 inhibitor known to evaluate the effect of UGT2B4 inhibition on soticlestat exposure. Furthermore, it was not possible to investigate the effect of UGT induction owing to the lack of specific and potent UGT inducers. However, rifampin has been reported to induce UGT2B4 and UGT1A9 in human hepatocytes (Soars et al., 2004; Gufford et al., 2018), possibly suggesting the minimal increase in M3 exposure observed in the rifampin study could be related to rifampin’s effect on UGT enzyme induction.

All AEs were mild or moderate when soticlestat was administered alone or with itraconazole, mefenamic acid, or rifampin. Of the few AEs that were considered by the investigator to be related to soticlestat, all were mild. Overall, safety findings were consistent with those from previous studies in healthy volunteers and patients with developmental and epileptic encephalopathies, including Dravet syndrome and Lennox–Gastaut syndrome. In all previous studies, AEs were mostly mild or moderate, especially for soticlestat doses equal to or below the maximum twice-daily dose (300 mg) under investigation in phase 3 studies. In the present study, TEAEs occurred at similar rates across study parts and treatment groups, which
is also aligned with findings from previous studies (Halford et al., 2021; Wang et al., 2021; Hahn et al., 2022; Wang et al., 2022; Yin et al., 2023).

In conclusion, soticlestat can be coadministered with strong CYP3A and UGT1A9 inhibitors. To ensure that soticlestat exposure remains within an acceptable range for efficacy, soticlestat is not recommended to be coadministered with strong CYP3A inducers (except for antiseizure medications, such as carbamazepine, phenobarbital and phenytoin, which did not affect soticlestat plasma total exposure in phase 2 studies). These findings were used to inform protocol development for subsequent phase 3 studies in patients with Dravet syndrome and Lennox–Gastaut syndrome taking CYP3A inhibitors or inducers or UGT1A9 inhibitors, and in those initiating treatment with CYP3A inhibitors or inducers or UGT1A9 inhibitors. In both DDI studies, soticlestat was well tolerated when administered with and without itraconazole, mfenamic acid, or rifampin as evidenced by evaluation of AEs, laboratory tests, vital signs, C-SSRS results, and ECG data.
Acknowledgments

The authors would like to thank the principal investigators Allen Hunt and Devinda Weeraratne and the study participants. Under the direction of the authors and funded by Takeda Pharmaceutical Company Limited, Dr R. Huntly PhD and Dr A. Jones PhD of Oxford PharmaGenesis, Oxford, UK, provided writing assistance for this publication, in compliance with current Good Publication Practice guidelines. Editorial assistance in formatting, proofreading, copy editing, and fact checking was also provided by Oxford PharmaGenesis.

Data Availability

The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants data supporting the results reported in this article, will be made available within three months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection and requirements for consent and anonymization.

Authorship Contributions

Participated in research design: W. Yin, C. Dong, A. Stevenson, V. Lloyd, M. Petrillo, M. Baratta, T. Hui, S. Han.

Performed data analysis: Clinical Pharmacology and Biostatistics.

Wrote or contributed to the writing of the manuscript: W. Yin, C. Dong, A. Stevenson, V. Lloyd, M. Petrillo, M. Baratta, T. Hui, S. Han.
References


Footnotes

a) This study was funded by the sponsor, Takeda Pharmaceutical Company Limited.

b) WY, CD, AS, VL, MB, TH, and SH are employees of Takeda Pharmaceuticals USA, Inc., and stockholders of Takeda Pharmaceutical Company Limited. MP has nothing to disclose.

c) Previously presented at the American Epilepsy Society Annual Meeting, December 2–6, 2022, Nashville, TN, USA.

d) Reprint requests: Wei Yin, Takeda Development Center Americas, Inc., 35 Landsdowne St, Cambridge, MA 02139, USA; Email: wei.yin@takeda.com
Figure legends

Fig. 1. Soticlestat plasma concentration–time curves in the presence or absence of (A) itraconazole, (B) mefenamic acid or (C) rifampin (semi-log scale).

SD, standard deviation.
### Tables

**TABLE 1** Baseline demographics

<table>
<thead>
<tr>
<th></th>
<th><strong>Itraconazole and mefenamic acid study</strong></th>
<th><strong>Rifampin study</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Part 1&lt;sup&gt;a&lt;/sup&gt; (n = 14)</strong></td>
<td><strong>Part 2&lt;sup&gt;b&lt;/sup&gt; (n = 14)</strong></td>
</tr>
<tr>
<td>Mean age (S.D.), years</td>
<td>40.7 (9.23)</td>
<td>40.2 (8.34)</td>
</tr>
<tr>
<td>Mean body weight (S.D.), kg</td>
<td>80.23 (11.879)</td>
<td>84.85 (12.211)</td>
</tr>
<tr>
<td>Mean BMI (S.D.), kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.300 (2.6527)</td>
<td>28.906 (2.9670)</td>
</tr>
<tr>
<td>Male, %</td>
<td>78.6</td>
<td>85.7</td>
</tr>
<tr>
<td>White, %</td>
<td>57.1</td>
<td>35.7</td>
</tr>
<tr>
<td>Black/African American, %</td>
<td>28.6</td>
<td>35.7</td>
</tr>
<tr>
<td>Not Hispanic or Latino, %</td>
<td>85.7</td>
<td>85.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effects of itraconazole on soticlestat PK.

<sup>b</sup>Effects of mefenamic acid on soticlestat PK.

BMI, body mass index; PK, pharmacokinetics; S.D., standard deviation.
TABLE 2 PK parameters of soticlestat, M-I, and M3 following itraconazole coadministration (part 1) in the itraconazole and mefenamic acid study

<table>
<thead>
<tr>
<th></th>
<th>Soticlestat alone (n = 14)</th>
<th>Soticlestat + itraconazole (n = 14)</th>
<th>Soticlestat alone (n = 14)</th>
<th>Soticlestat + itraconazole (n = 14)</th>
<th>Soticlestat alone (n = 14)</th>
<th>Soticlestat + mefenamic acid (n = 14)</th>
<th>Soticlestat alone (n = 14)</th>
<th>Soticlestat + mefenamic acid (n = 14)</th>
<th>Soticlestat alone (n = 14)</th>
<th>Soticlestat + mefenamic acid (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>t_{max}, h</strong></td>
<td>0.5 (0.5, 0.8)</td>
<td>0.5 (0.5, 1.0)</td>
<td>0.8 (0.5, 1.5)</td>
<td>0.7 (0.5, 1.5)</td>
<td>0.9 (0.5, 1.5)</td>
<td>0.5 (0.5, 0.6)</td>
<td>0.8 (0.5, 1.5)</td>
<td>0.5 (0.5, 0.8)</td>
<td>0.8 (0.5, 0.8)</td>
<td>0.8 (0.5, 0.8)</td>
</tr>
<tr>
<td><strong>C_{max}, ng/ml</strong></td>
<td>1310 (80.2)</td>
<td>1527 (65.5)</td>
<td>259.5 (44.1)</td>
<td>23580 (26.4)</td>
<td>24670 (14.7)</td>
<td>1414 (58.4)</td>
<td>1517 (55.8)</td>
<td>252.4 (35.2)</td>
<td>297.8 (30.9)</td>
<td>26020 (21.0)</td>
</tr>
<tr>
<td><strong>AUC_{last}, ng·h/ml</strong></td>
<td>1391 (54.9)</td>
<td>1697 (45.3)</td>
<td>511.6 (32.7)</td>
<td>52490 (16.8)</td>
<td>63180 (13.2)</td>
<td>1423 (41.6)</td>
<td>1528 (46.2)</td>
<td>511.1 (32.7)</td>
<td>612.1 (26.8)</td>
<td>57160 (24.0)</td>
</tr>
<tr>
<td><strong>AUC_{∞}, ng·h/ml</strong></td>
<td>1483 (56.1)^a</td>
<td>1914 (42.8)^a</td>
<td>538.0 (31.4)^b</td>
<td>52760 (16.8)</td>
<td>63460 (13.2)</td>
<td>1533 (42.2)^a</td>
<td>1594 (45.6)^a</td>
<td>520.6 (31.8)^b</td>
<td>590.0 (21.8)^b</td>
<td>57520 (24.0)</td>
</tr>
<tr>
<td><strong>AUC_{extrap%}, ng·h/ml</strong></td>
<td>1.3 ± 0.6^a</td>
<td>1.2 ± 0.7^a</td>
<td>1.7 ± 1.2^a</td>
<td>7.4 ± 4.9</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>1.0 ± 0.7^a</td>
<td>2.1 ± 2.5^a</td>
<td>1.8 ± 1.2^a</td>
<td>2.8 ± 2.4^a</td>
</tr>
<tr>
<td><strong>t_{1/2z}, h</strong></td>
<td>5.3 ± 1.7^a</td>
<td>5.0 ± 1.5^a</td>
<td>3.0 ± 1.4^a</td>
<td>2.3 ± 1.4</td>
<td>5.1 ± 0.9</td>
<td>4.6 ± 0.9</td>
<td>4.7 ± 2.4^a</td>
<td>5.8 ± 2.5^a</td>
<td>3.0 ± 1.6</td>
<td>4.5 ± 3.5^a</td>
</tr>
<tr>
<td><strong>CL/F, l/h</strong></td>
<td>232.3 ± 143.1^a</td>
<td>169.6 ± 76.2^a</td>
<td>n/a</td>
<td>n/a</td>
<td>211.4 ± 91.1^a</td>
<td>206.5 ± 105.7^a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>V_{z}/F, l</strong></td>
<td>1720 ± 1191.7^a</td>
<td>1201 ± 543.7^a</td>
<td>n/a</td>
<td>n/a</td>
<td>1447 ± 865.7^a</td>
<td>1877 ± 1445.0^a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
\(^a\)AUC\(_\infty\), AUC\(_{extrap}\%), t_{1/2z}, CL/F and V_{z/F} could not be calculated for two participants following soticlestat alone and five participants following soticlestat + itraconazole because the terminal disposition phase could not be characterized.

\(^b\)AUC\(_\infty\), AUC\(_{extrap}\%), t_{1/2z}, CL/F and V_{z/F} could not be calculated for one participant following soticlestat alone because the terminal disposition phase could not be characterized.

AUC\(_\infty\), area under the concentration–time curve from time 0 to infinity; AUC\(_{extrap}\%) AUC\(_\infty\) expressed as a percentage; AUC\(_{last}\), area under the concentration–time curve from time 0 to time of last quantifiable concentration; CL/F apparent clearance after extravascular administration; C\(_{max}\), maximum observed concentration; n/a, not applicable; PK, pharmacokinetics; t\(_{1/2z}\), terminal disposition phase half-life; t\(_{max}\) time of first occurrence of C\(_{max}\); V\(_{z/F}\), apparent volume of distribution during the terminal disposition phase after extravascular administration.
TABLE 3 Effects of itraconazole (part 1) or mefenamic acid (part 2) coadministration on the PK of soticlestat, M-I, and M3 in the itraconazole and M mefenamic acid A study

<table>
<thead>
<tr>
<th></th>
<th>Part 1: effects of itraconazole on soticlestat PK</th>
<th>Part 2: effects of mefenamic acid on soticlestat PK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric LSM GMR, % (90% CI)</td>
<td>Geometric LSM GMR, % (90% CI)</td>
</tr>
<tr>
<td>Soticlestat +</td>
<td>Soticlestat alone (n)</td>
<td>Soticlestat + mefenamic acid alone (n)</td>
</tr>
<tr>
<td>itraconazole (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soticlestat</td>
<td>1527 (14)</td>
<td>1517 (14)</td>
</tr>
<tr>
<td>M-I</td>
<td>27.85 (14)</td>
<td>297.8 (14)</td>
</tr>
<tr>
<td>M3</td>
<td>24670 (14)</td>
<td>22940 (14)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soticlestat</td>
<td>1697 (14)</td>
<td>1528 (14)</td>
</tr>
<tr>
<td>M-I</td>
<td>65.37 (14)</td>
<td>612.1 (14)</td>
</tr>
<tr>
<td>M3</td>
<td>63180 (14)</td>
<td>52850 (14)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;last&lt;/sub&gt;, ng*h/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soticlestat</td>
<td>1697 (14)</td>
<td>1528 (14)</td>
</tr>
<tr>
<td>M-I</td>
<td>65.37 (14)</td>
<td>612.1 (14)</td>
</tr>
<tr>
<td>M3</td>
<td>63180 (14)</td>
<td>52850 (14)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt;, ng*h/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soticlestat</td>
<td>1833 (9)</td>
<td>1541 (9)</td>
</tr>
<tr>
<td>M-I</td>
<td>70.66 (14)</td>
<td>609.3 (12)</td>
</tr>
<tr>
<td>M3</td>
<td>63460 (14)</td>
<td>53270 (14)</td>
</tr>
</tbody>
</table>

AUC<sub>∞</sub>, area under the concentration–time curve from time 0 to infinity; AUC<sub>last</sub>, area under the concentration–time curve from time 0 to time of last quantifiable concentration; CI, confidence interval; C<sub>max</sub>, maximum observed concentration; GMR, geometric mean ratio; LSM, least-squares mean; PK, pharmacokinetics.
TABLE 4 Summary of TEAEs

<table>
<thead>
<tr>
<th>Study</th>
<th>Soticlestat alone (n = 14)</th>
<th>Itraconazole alone (n = 14)</th>
<th>Soticlestat + Itraconazole (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole and mefenamic acid study – part 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with TEAEs, n (%)</td>
<td>1 (7.1)</td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Number of TEAEs</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Serious TEAEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TEAEs leading to study discontinuation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Soticlestat alone (n = 14)</th>
<th>Mefenamic acid alone (n = 14)</th>
<th>Soticlestat + mefenamic acid (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole and mefenamic acid study – part 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with TEAEs, n (%)</td>
<td>1 (7.1)</td>
<td>0</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Number of TEAEs</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Serious TEAEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TEAEs leading to study discontinuation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Soticlestat alone (n = 15)</th>
<th>Rifampin alone (n = 14)</th>
<th>Soticlestat + rifampin (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with TEAEs, n (%)</td>
<td>2 (13.3)</td>
<td>1 (7.1)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Number of TEAEs</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Serious TEAEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TEAEs leading to study discontinuation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Participants may appear in more than one category in each study.
TEAEs, treatment-emergent adverse events.
### TABLE 5 PK parameters of soticlestat, M-I, and M3 in the rifampin study

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Soticlestat Soticlestat + M-I Soticlestat + M3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soticlestat alone</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
</tr>
<tr>
<td>(t_{\text{max}}), h</td>
<td>0.5 (0.2, 0.8)</td>
</tr>
<tr>
<td>(C_{\text{max}}), ng/ml</td>
<td>1364 (63.6)</td>
</tr>
<tr>
<td>(AUC_{\text{last}}), ng·h/ml</td>
<td>1290 (52.9)</td>
</tr>
<tr>
<td>(AUC_{\infty}), ng·h/ml</td>
<td>1448 (47.0)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(AUC_{\text{extrap}}%), ng·h/ml</td>
<td>2.1 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(t_{1/2z}), h</td>
<td>6.7 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(CL/F), L/h</td>
<td>226.0 ± 96.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(V_z/F), L</td>
<td>2139 ± 1794.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\(t_{\text{max}}\) values are presented as median (minimum, maximum). \(C_{\text{max}}\), \(AUC_{\text{last}}\), and \(AUC_{\infty}\) values are presented as geometric mean (geometric per cent coefficient of variation). \(AUC_{\text{extrap}}\%\), \(t_{1/2z}\), \(CL/F\) and \(V_z/F\) values are presented as arithmetic mean ± standard deviation.

\(<sup>a</sup>\)AUC\(_{\infty}\), AUC\(_{\text{extrap}}\%\), \(t_{1/2z}\), \(CL/F\) and \(V_z/F\) could not be calculated for two participants following soticlestat alone and three participants following soticlestat + rifampin because the terminal disposition phase could not be characterized.

AUC\(_{\infty}\), area under the concentration–time curve from time 0 to infinity; AUC\(_{\text{extrap}}\%\), AUC\(_{\infty}\) expressed as a percentage; AUC\(_{\text{last}}\), area under the concentration–time curve from time 0 to time of last quantifiable concentration; CL/F apparent clearance after extravascular administration; \(C_{\text{max}}\), maximum observed concentration; n/a, not applicable; PK, pharmacokinetics; \(t_{1/2z}\), terminal disposition phase half-life; \(t_{\text{max}}\), time of first occurrence of \(C_{\text{max}}\); \(V_z/F\), apparent volume of distribution during the terminal disposition phase after extravascular administration.
TABLE 6 Effect of rifampin coadministration on the PK of soticlestat, M-I, and M3 in the rifampin study

<table>
<thead>
<tr>
<th></th>
<th>Geometric LSM</th>
<th>GMR, % (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soticlestat + rifampin (n)</td>
<td>Soticlestat alone (n)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, ng/ml</td>
<td>179.4 (14)</td>
<td>1364 (15)</td>
</tr>
<tr>
<td></td>
<td>629.8 (14)</td>
<td>236.7 (15)</td>
</tr>
<tr>
<td></td>
<td>16670 (14)</td>
<td>25050 (15)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;last&lt;/sub&gt;, ng*h/ml</td>
<td>192.4 (14)</td>
<td>1290 (15)</td>
</tr>
<tr>
<td></td>
<td>869.3 (14)</td>
<td>475.2 (15)</td>
</tr>
<tr>
<td></td>
<td>29930 (14)</td>
<td>51230 (15)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt;, ng*h/ml</td>
<td>220.5 (11)</td>
<td>1343 (13)</td>
</tr>
<tr>
<td></td>
<td>877.2 (14)</td>
<td>485.2 (15)</td>
</tr>
<tr>
<td></td>
<td>30100 (14)</td>
<td>51560 (15)</td>
</tr>
</tbody>
</table>

AUC<sub>∞</sub>, area under the concentration–time curve from time 0 to infinity; AUC<sub>last</sub>, area under the concentration–time curve from time 0 to time of last quantifiable concentration; CI, confidence interval; C<sub>max</sub>, maximum observed concentration; GMR, geometric mean ratio; LSM, least-squares mean; PK, pharmacokinetics.
Figure 1

(A) Arithmetic mean + SD plasma soticlestat concentration (ng/ml) vs. Nominal hours after dosing

(B) Arithmetic mean + SD plasma soticlestat concentration (ng/ml) vs. Nominal hours after dosing

(C) Arithmetic mean + SD plasma soticlestat concentration (ng/ml) vs. Nominal hours after dosing

Legend:
- Soticlestat alone
- Soticlestat + itraconazole
- Soticlestat alone
- Soticlestat + mefenamic acid
- Soticlestat alone
- Soticlestat + rifampin

This article has not been copyedited and formatted. The final version may differ from this version.
Supplementary Materials

Effects of Strong Inhibition of CYP3A and UGT1A9 and Strong Induction of CYP3A on the Pharmacokinetics, Safety, and Tolerability of Soticlestat: Two Drug–Drug Interaction Studies in Healthy Volunteers

Wei Yin, Cheng Dong, Annette Stevenson, Valerie Lloyd, Marco Petrillo,* Mike Baratta, Tom Hui, and Steve Han


*At the time the study was conducted.
Supplementary Fig. 1. Study design

(A) Study 1 – Part 1

Screening

Within the 28 days before first dosing

Period 1

Check-in and pre-dose assessments

Day –1

PK sampling and study assessments

Day 1

Days 2–5a

Soticlestat (300 mg)

Washout

Period 2

Study assessments

Days 1–4

Day 5

Days 6–11

Day 12

Itraconazole (200 mg)

Itraconazole (200 mg) + soticlestat (300 mg)

Itraconazole (200 mg)

Study exitb

Follow-up

15 (± 2) days after last soticlestat dose

Study 1 – Part 2

Screening

Within the 28 days before first dosing

Period 1

Check-in and pre-dose assessments

Day –1

PK sampling and study assessments

Day 1

Days 2–5a

Soticlestat (300 mg)

Washout

Period 2

Study assessments

Day 1a

Day 2

Days 3–7

Day 8

MA (600 mg)

MA (250 mg) + soticlestat (300 mg)

MA (250 mg)

Study exitb

Follow-up

15 (± 2) days after last soticlestat dose

(B) Study 2

Screening

Within the 28 days before first dosing

Period 1

Check-in and pre-dose assessments

Day –1

PK sampling and study assessments

Day 1

Days 2–5a

Soticlestat (300 mg)

Washout

Period 2

Study assessments

Days 1–10

Day 11

Days 12–14d

Rifampin (600 mg) + soticlestat (300 mg)

Rifampin (600 mg)

Rifampin (600 mg)

Follow-up

15 (± 2) days after last soticlestat dose

aDay 1 of period 2 was also considered to be day 5 of period 1.
bStudy exit was defined as the end of the last treatment period.
cParticipants received mefenamic acid every 6 hours. The initial dose on day 1 was 500 mg and all subsequent doses were 250 mg.
dThe last dose of rifampin was administered on day 13.

MA, mefenamic acid; PK, pharmacokinetic.
Supplementary Fig. 2. Individual plots for soticlestat plasma concentration–time curves in the presence or absence of itraconazole (semi-log scale).
Supplementary Fig. 3. Individual plots for soticlestat plasma concentration–time curves in the presence or absence of mefenamic acid (semi-log scale).
Supplementary Fig. 4. Individual plots for soticlestat plasma concentration–time curves in the presence or absence of rifampin (semi-log scale).
Supplementary Methods

Bioanalytical Methods of Soticlestat and M-I

Blood samples were collected in chilled tubes containing potassium ethylenediaminetetraacetic acid and centrifuged within 60 minutes of collection. Following centrifugation, aliquots of harvested plasma were added to separate vials and stored at −70°C. Following thawing, a 50 μl plasma aliquot was added to a 96-well plate followed by 20 μl of internal standard solution (1000 ng/ml of soticlestat-d5 and M-I-d5 in acetonitrile/10 mM ammonium formate [60/40] [v/v], respectively). The solution containing analytes and internal standards in diluted plasma was added to an Oasis HLB 30 mg, 1 ml solid-phase extraction plate that was previously conditioned with 1 ml of acetonitrile followed by 1 ml of 10 mM ammonium formate. The solid-phase extraction plate was washed with 1 ml of a 10% methanol solution in deionized water followed by elution with 1 ml of acetonitrile into a 2 ml 96-well plate. The eluent was evaporated to dryness under a stream of nitrogen at a temperature of 40°C. The extract was reconstituted with 500 μl of acetonitrile/10 mM ammonium formate (60/40) (v/v) in a 1 ml 96-well plate, capped and vortexed for 10 seconds. A 50 μl aliquot of this solution was removed and diluted with 450 μl of acetonitrile/10 mM ammonium formate (60/40) (v/v) capped and vortexed for 10 seconds.

A 1 μl injection was performed on an API-5500 mass spectrometer (SCIEX, Framingham, MA, USA) equipped with a Waters Acquity UPLC system (Agilent Technologies, Santa Clara, CA, USA). A reverse-phase gradient method running at a flow rate of 0.8 ml/min on a Phenomenex Synergi MAX-RP, 4.6 × 50 mm, 2.5 μm column (Phenomenex, Torrance, CA, USA) provided soticlestat and M-I retention times of 1.02 and 0.80 (± 0.1) minutes, respectively. The gradient elution employed mobile phases of (95:5) (v/v) 10 mM ammonium formate: acetonitrile (A); and (5:95) (v/v) 10 mM ammonium formate: acetonitrile (B). Soticlestat, M-I, and their respective internal standards were ionized under a positive ion spray mode and detected through multiple reaction monitoring of mass transition pairs at m/z (soticlestat/internal standard) 374.0→128.0 and 379.0→183.0, (M-I/internal standard) 390.0→199.0 and 395.9→199.0, respectively. Calibration curves for each analyte were established using
standards, and the peak area ratios of the analyte against the isotopically labeled internal standard were used to quantify samples. Linearity was achieved for the soticlestat and M-I concentration range of 1.00–2000 ng/mL with quality control samples ranging from 3.00 to 1600 ng/ml. Incurred sample reanalysis of plasma samples met acceptance criteria.

Bioanalytical Methods of M3

Blood samples were collected in chilled tubes containing potassium ethylenediaminetetraacetic acid and centrifuged within 60 minutes of collection. Following centrifugation, aliquots of harvested plasma were added to separate vials and stored at −70°C. Following thawing, a 50 μl plasma aliquot was added to a 96-well plate followed by 350 μl of 1% formic acid in water solution and 50 μl of internal standard solution (1000 ng/ml of M3-d4 in acetonitrile/10 mM ammonium formate (80/20, v/v), respectively). The solution containing the analyte and internal standard in diluted plasma was added to an Evolute CX 30 mg solid-phase extraction plate that was previously conditioned with 0.5 ml of methanol followed by 0.5 ml of 1% formic acid in water solution. The solid-phase extraction plate was washed with 0.5 ml of a 1% formic acid in water solution and 0.5 ml of a 5% ammonia solution in deionized water followed by elution with 0.5 ml of methanol into a 2 ml 96-well plate. The eluent was evaporated to dryness under a stream of nitrogen at a temperature of 40°C. The extract was reconstituted with 500 μl of 10 mM ammonium acetate/acetonitrile (90/10, v/v) in a 1 ml 96-well plate, capped and vortexed for 10 seconds.

A 1 μl injection was performed on an API-5500 mass spectrometer (SCIEX, Framingham, MA, USA) equipped with a Waters Acquity UPLC system (Agilent Technologies, Santa Clara, CA, USA). A reverse-phase gradient method running at a flow rate of 0.8 ml/min on a Phenomenex Synergi MAX-RP, 2.0 × 50 mm, 2.5 μm column (Phenomenex, Torrance, CA, USA) provided a M3 retention time of 1.15 (± 0.1) minutes. The gradient elution employed mobile phases of (95:5) (v/v) 10 mM ammonium acetate: acetonitrile (A); and (5:95) (v/v) 10 mM ammonium acetate: acetonitrile (B). M3 and its internal standard were ionized under a positive ion spray mode and detected through multiple reaction monitoring of mass transition pairs at
m/z (M3/internal standard) 550.2→374.2 and 554.2→378.4. Calibration curves for each analyte were established using standards, and the peak area ratios of the analyte against the isotopically labeled internal standard were used to quantify samples. Linearity was achieved for M3 over the concentration range of 10.0–20 000 ng/ml, with quality control samples ranging from 30.0 to 16000 ng/ml. Incurred sample reanalysis of plasma samples met acceptance criteria.