2. SYNOPSIS

Study Title

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, SINGLE ASCENDING DOSE AND MULTIPLE ASCENDING DOSE STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF REC-3964 IN HEALTHY SUBJECTS

Study Codes

Sponsor code : REC-3964-101
ICON code : RCU2110A-2110AX
EudraCT number : 2022-002403-38

Sponsor

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Publication : None at time of writing this report

Study Period : Date of first screening to last follow-up: 17 Aug 2022 to 09 May 2023

Clinical Phase : Phase 1

Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of single- and multiple-ascending doses of REC-3964 administered orally to healthy subjects.	Primary safety endpoints of the study including: • clinical laboratory assessments (hematology, chemistry, coagulation, and urinalysis) • 12-lead electrocardiograms (ECGs) • cardiac telemetry • vital sign measurements • physical examinations • incidence of adverse events (AEs) and serious adverse events (SAEs).

Objectives	Endpoints
Secondary	
• To characterize the pharmacokinetics (PK) of REC-3964 and its enantiomer, REC-3974, in plasma and urine following single and multiple oral doses of REC-3964 in healthy subjects.	**Part A (Single Ascending Doses)** • Plasma PK parameters for REC-3964 and REC-3974 including, but not limited to: ○ C_{max}, T_{max}, AUC_{last}, AUC_{inf}, and $T_{1/2}$; enantiomer ratios (ER) of REC-3974 to REC-3964 (ER[C_{max}] and ER[AUC_{last}]); and CL/F and V_z/F (REC-3964 only). • Urine PK parameters for REC-3964 and REC-3974 including, but not limited to: ○ Ae_{0-t}, Fe (REC-3964 only), and CL_R.
	Part B (Multiple Ascending Doses) • Plasma PK parameters for REC-3964 and REC-3974 including, but not limited to: ○ Day 1: C_{max}, T_{max}, C_{trough}, AUC_{tau}; and ER(C_{max}) and ER(AUC_{tau}) ○ Days 2, 3, 4, 7, 10, and 13: C_{trough} ○ Day 14: C_{max}, T_{max}, C_{trough}, AUC_{tau}, AUC_{inf}, $T_{1/2}$; accumulation index for C_{max} and AUC_{tau} (AI[C_{max}] and AI[AUC_{tau}]); ER(C_{max}) and ER(AUC_{tau}); and CL/F and V_z/F (REC-3964 only). • Urine PK parameters for REC-3964 and REC-3974 including, but not limited to: ○ Day 1 and Day 14: Ae_{tau}, Fe (REC-3964 only), and CL_R.
Exploratory	
• To investigate the metabolite profile of REC-3964 in plasma following single and multiple oral doses of REC-3964 in healthy subjects.	• Metabolite profile of REC-3964 in plasma. [a]
• To investigate the potential genetic variants influencing the PK of REC-3964 in healthy subjects.	• Potential genotyping analysis to correlate individual subject exposures (or other variables) with specific genetic variants. [a]
• To investigate potential blood biomarkers of REC-3964 activity following single and multiple oral doses in healthy subjects.	• Potential future biomarker analysis from collected blood samples. [a]
• To evaluate changes in cytochrome P450 3A (CYP3A) activity following multiple oral doses of REC-3964 in healthy subjects.	• Plasma 4β-hydroxycholesterol/cholesterol ratio (Part B only). [b]
• To estimate the fraction of unbound REC-3964 in plasma following single oral doses of REC-3964 in healthy subjects.	• Fraction of unbound REC-3964 in plasma on Day 1 (Part A only). [a]

[a] If evaluated (for potential future analyses), results will be reported separately from this clinical study report.
[b] Changes in CYP3A activity were evaluated based on 4β-hydroxycholesterol levels in plasma instead of the 4β-hydroxycholesterol/cholesterol ratio.

Design and Treatments

This was a Phase 1, single-center, randomized, double-blind, placebo-controlled, first-in-human study to investigate the safety, tolerability, and PK of REC-3964 in healthy subjects after single and multiple oral doses of REC-3964. The study consisted of 2 parts, with a single ascending dose (SAD; Part A) and a multiple ascending dose (MAD; Part B) design. Escalating single and multiple doses of REC-3964 were selected by a chartered dose escalation committee (DEC) after review of the safety and available PK data from the previous cohort(s).

Part A

Part A consisted of 5 sequential cohorts in healthy subjects (aged ≥18 to ≤65 years; Cohorts A1 to A5) and 1 cohort with healthy elderly subjects (>65 years of age; Cohort A7). Furthermore, 1 optional cohort (Cohort A6) could evaluate a dose that was lower than, higher than, or equal to a previously administered dose. In each cohort, 8 subjects were randomized in a 3:1 ratio to receive either REC-3964 (6 subjects) or placebo (2 subjects).

Each cohort included 2 sentinel subjects (1 received placebo and 1 received REC-3964). The remaining subjects in each cohort were dosed at least 24 hours after the dosing of the initial 2 sentinel subjects, provided that their safety and tolerability results were deemed acceptable by the Investigator.

For an overview of the planned single-dose treatments administered, see below:

Cohort A1: Single oral dose of 50 mg REC-3964 (n=6) or placebo (n=2)
Cohort A2: Single oral dose of 100 mg REC-3964 (n=6) or placebo (n=2)
Cohort A3: Single oral dose of 300 mg REC-3964 (n=6) or placebo (n=2)
Cohort A4: Single oral dose of 600 mg REC-3964 (n=6) or placebo (n=2)
Cohort A5: Single oral dose of 1200 mg REC-3964 (n=6) or placebo (n=2)
Cohort A7[a]: Single oral dose of 600 mg REC-3964 (n=6) or placebo (n=2)

[a] In healthy elderly subjects aged >65 years (instead of subjects aged ≥18 to ≤65 years).
Note: the optional Cohort A6 was not dosed.

Part B

Part B consisted of 4 sequential cohorts (Cohorts B1 to B4) in healthy subjects aged ≤65 years. One optional cohort (Cohort B5) could evaluate a dose that was lower than, higher than, or equal to a previously administered dose in healthy (elderly) subjects. In each cohort, 10 healthy subjects were planned to be randomized in a 4:1 ratio to receive either REC-3964 (8 subjects) or placebo (2 subjects).

In Part B, REC-3964 or placebo was administered for 14 days in once daily (QD), twice daily (BID; every 12 hours), or three times daily (TID; every 8 hours) dose regimens. Dosing of the first MAD cohort in Part B (Cohort B1) was initiated after completion of Cohort A3 in the SAD part as per protocol. The doses and dose regimens of REC-3964 in Part B were selected by the DEC.

For an overview of the planned multiple-dose treatments, see below:

Cohort B1: Multiple oral doses of 100 mg REC-3964 (n=8) or placebo (n=2) administered TID for 14 days [a]
Cohort B2: Multiple oral doses of 300 mg REC-3964 (n=8) or placebo (n=2) administered BID for 14 days
Cohort B3: Multiple oral doses of 500 mg REC-3964 (n=8) or placebo (n=2) administered BID for 14 days
Cohort B4: Multiple oral doses of 900 mg REC-3964 (n=8) or placebo (n=2) administered QD for 14 days

[a] For the actual number of subjects dosed, see “Subject Disposition” below.

Note: the optional Cohort B5 was not dosed.

Study Schedule

Screening	: Between Day -28 and Day -2 (admission).
Treatment period	: One period in the clinic each being from Day -2 (admission) until discharge on Day 3 (Part A) or Day 16 (Part B), after completion of the safety and PK assessments until 48 hours after (the last) study drug administration on Day 1 (Part A) or Day 14 (Part B).
End-of-study visit	: Day 7 (±1 day) in Part A or Day 20 (±2 days) in Part B.

Subjects

A total of 88 healthy subjects were planned to be enrolled in the study (excluding optional cohorts), including:

Part A (6 cohorts)	: 48 healthy male and/or female subjects, including 8 healthy elderly subjects
Part B (4 cohorts)	: 40 healthy male and/or female subjects

Main Criteria for Inclusion

Subjects	: Healthy male and/or female subjects
Age	: ≥18 to ≤65 years. In Cohort A7, the healthy elderly subjects were aged >65 years (note: there was no upper limit for the subject's age).
Weight	: ≥50 kg
Body mass index (BMI)	: 18 to 32 kg/m^2, inclusive

Study Drug

Active Medication

Active substance	: REC-3964
Activity	: Inhibition of *Clostridium difficile* toxin effects
In development for	: *Clostridium difficile* infection
Strength	: 25 mg – 200 mg
Dosage form	: Swedish orange opaque size 00 hydroxypropylmethylcellulose oral capsule(s)
Manufacturer	: ICON Manufacturing (PRA Group BV)
Batch number	: JR-C201019002-FPF22001 (drug substance)

Placebo

Substance	: Microcrystalline cellulose
Activity	: Not applicable
Strength	: Not applicable
Dosage form	: Swedish orange opaque size 00 hydroxypropylmethylcellulose oral capsule(s)
Manufacturer	: ICON Manufacturing (PRA Group BV)
Batch numbers	: See manufacturing batch records (available on file).

The batch numbers of the investigational drug products (REC-3964 and placebo) can be found in the manufacturing batch records, which are available on file.

Statistical Methods

Safety parameters : Descriptive statistics

PK parameters : Descriptive statistics for all relevant PK parameters: number of subjects, mean, standard deviation, median, minimum, maximum, coefficient of variation, geometric mean, and geometric coefficient of variation.

For Part A, dose proportionality was explored by comparing the PK parameters (C_{max}, AUC_{last}, and AUC_{inf}) of REC-3964 across the evaluated dose levels of REC-3964. Statistical analysis of dose proportionality was performed using a power model (primary analysis) and analysis of variance (ANOVA) method (secondary analysis) based on these PK parameters.

The power model addressed dose proportionality in terms of point estimates and 90% confidence intervals (CIs) of the statistical model parameters (slopes).

The ANOVA model estimated the ratio of geometric least-squares means (GLSMs) and corresponding 90% CIs for dose-normalized and natural logarithmically transformed PK parameters at the tested dose levels, compared to the reference dose level (ie, the 50 mg starting dose level in Part A).

Exploratory parameters : Descriptive statistics of 4β-hydroxycholesterol (4β-OH-cholesterol) levels

Results

Subject Disposition

A total of 296 subjects signed the informed consent form at screening, and 90 of these subjects were enrolled in the study (Table S1). All enrolled subjects were randomized and dosed.

- In Part A, 48 subjects were randomized in a 3:1 ratio to receive either REC-3964 or placebo as planned. All randomized subjects were included in the safety set. A total of 36 subjects received a single dose of REC-3964, and all of them were included in the PK set. All 48 subjects completed Part A of the study as per protocol.
- In Part B, 42 subjects were randomized in a 4:1 ratio to receive either REC-3964 or placebo. All randomized subjects were included in the safety set. A total of 34 subjects received at least 1 dose of REC-3964, and all of them were included in the PK set. Forty subjects completed Part B of the study as per protocol and 2 subjects in Cohort B1 were early discontinued and replaced:
  - One subject was withdrawn from the study after the morning dose of 100 mg REC-3964 TID on Day 8 due to unacceptable behavior toward the study staff.
  - One subject was withdrawn from the study after the morning dose of 100 mg REC-3964 TID on Day 3 due to poor venous accessibility.

  No special data handling was required for missing PK concentrations of the 2 subjects who were discontinued early.

Table S1 Disposition of Subjects and Analysis Sets

	Number of Subjects	
Screened subjects (who signed the informed consent form at screening)	296	
Screening failures and subjects approved but not enrolled	206	
Subjects enrolled	90	
	Part A (N=48)	**Part B (N=42)**
Enrolled set [a]	48	42
Randomized	48 (100.0)	42 (100.0)
Safety set	48 (100.0)	42 (100.0)
Pharmacokinetic set	36 (75.0)	34 (81.0)
Completed subjects	48 (100.0)	40 (95.2)
Discontinued subjects; reasons:	0	2 (4.8)
Other [b]	0	2 (4.8)

Note: Percentages (%) are based on the number of subjects in the enrolled set.

[a] There were no screen failures after enrollment, defined as participants who were admitted on Day -2 but not randomized on Day 1.

[b] Two subjects who received 100 mg REC-3964 three times daily in Cohort B1 were discontinued, including one subject due to unacceptable behavior toward the study staff and one subject due to poor venous accessibility.

Demographics

In Part A, a total of 24 (50.0%) female and 24 (50.0%) male subjects between 18 and 74 years of age and with a BMI between 18.2 and 28.7 kg/m^2 participated in in Cohorts A1 to A7. A total of 44 (91.7%) subjects were White, 3 (6.3%) subjects were Asian, and 1 (2.1%) subject was of other race. Two (4.2%) subjects were of Hispanic or Latino ethnicity. Within Cohort A7, a total of 5 (83.3%) female and 1 (16.7%) male subjects between 67 and 74 years of age and with a BMI between 20.7 and 28.3 kg/m^2 received REC-3964; all of these healthy elderly subjects were White and none were of Hispanic or Latino ethnicity.

In Part B, a total of 10 (23.8%) female and 32 (76.2%) male subjects between 20 and 64 years of age and with a BMI between 20.0 and 30.6 kg/m^2 participated in Cohorts B1 to B4. A total of 38 (90.5%) subjects were White, 1 (2.4%) subject was Asian, 1 (2.4%) subject was Black or African American, and 2 (4.8%) subjects were of other race. None of the subjects were of Hispanic or Latino ethnicity.

The mean age and BMI were comparable across cohorts that included subjects aged ≤65 years, except that subjects receiving REC-3964 in Cohort A5 of Part A were slightly younger (24.2 years) than those in Cohorts A1 to A4 (mean range: 30.8 to 38.0 years).

Safety

Overall Tolerability

No deaths, SAEs, or discontinuations due to AEs were reported during the study. In both study parts, the percentage of subjects experiencing treatment-emergent adverse events (TEAEs) was comparable between subjects who received REC-3964 and placebo. There was no association identified between the dose of REC-3964 and percentage of subjects experiencing TEAEs. All TEAEs were of mild intensity.

Single oral doses of REC-3964 were well tolerated by healthy subjects at doses from 50 mg to 1200 mg, including a 600 mg dose of REC-3964 administered to healthy elderly subjects (aged >65 years). Also, multiple oral doses of REC-3964 were well tolerated by healthy subjects at total daily doses from 300 mg to 1000 mg for 14 days (in dose regimens of 100 mg TID, 300 mg BID, 500 mg BID, or 900 mg QD).

No clinically relevant safety laboratory, 12-lead ECG, telemetry monitoring, vital signs, or physical examination findings were reported.

Part A (Single Ascending Doses)
A total of 21 TEAEs were reported by 15 (41.7%) of the 36 subjects who received REC-3964 and 6 TEAEs were reported by 4 (33.3%) of the 12 subjects who received placebo. For REC-3964, the most frequently reported TEAE (ie, reported by >10% subjects) by Medical Dictionary for Regulatory Activities (MedDRA) preferred term (PT) was contact dermatitis (due to Holter electrode patches; by 4 [11.1%] subjects each). For placebo, only diarrhea was reported more than once (by 2 [16.7%] subjects). At a single dose of 600 mg REC-3964, TEAEs were reported at a comparable rate between subjects aged ≤65 years and elderly subjects aged >65 years (2 [33.3%] subjects each).

One TEAE of fatigue experienced by a subject who received 100 mg REC-3964 and 1 TEAE of diarrhea experienced by a subject who received placebo were considered by the Investigator to be related to the study drug. All TEAEs were of Grade 1 (mild) severity, transient, and resolved without sequelae.

Part B (Multiple Ascending Doses)
A total of 45 TEAEs were reported by 21 (61.8%) of the 34 subjects who received REC-3964 and 17 TEAEs were reported by 6 (75.0%) of the 8 subjects who received placebo. For REC-3964, the most frequently reported TEAEs (ie, reported by >10% subjects) by MedDRA PT were fatigue and headache (by 6 [17.6%] subjects each), medical device site irritation (by 5 [14.7%] subjects), and abdominal distension and catheter site irritation (by 4 [11.8%] subjects each). For placebo, the most frequently reported TEAEs were abdominal distension (by 3 [37.5%] subjects), and diarrhea, fatigue, and dry skin (by 2 [25.0%] subjects each).

A higher frequency of subjects reporting TEAEs was observed for the 100 mg TID dose regimen (daily dose of 300 mg; 8 [80%] subjects) than for 300 mg BID (daily dose of 600 mg; 4 [50.0%] subjects). This discrepancy was deemed to be related to the nighttime protocol procedures resulting in a lack of sleep and associated symptoms for subjects who received the TID dose regimen.

For 4 (11.8%) subjects who received REC-3964, the following TEAEs were considered by the Investigator to be related to the study drug: abdominal distension (reported by 1 subject each at a dose of 100 mg TID, 300 mg BID, and 500 mg BID) and flatulence (by 1 subject at 100 mg TID). Abdominal distension was also considered to be related to the study drug for 2 (25.0%) subjects who received placebo. All TEAEs were of Grade 1 (mild) severity, transient, and resolved without sequelae.

Pharmacokinetics
Plasma PK in Part A (Single Ascending Doses)
After administration of single oral doses of 50 to 1200 mg REC-3964 under fasted conditions in subjects aged ≤65 years, the median T_{max} of REC-3964 ranged between 0.77 and 2.00 hours (Table S2). The elimination of REC-3964 appeared to be biphasic. After reaching a peak concentration, REC-3964 concentrations decreased quickly during the initial elimination phase followed by a slower terminal elimination phase (geometric mean $T_{1/2}$ ranging from 6.93 to 9.52 hours). The geometric mean CL/F (range: 14.7 to 20.1 L/h) and geometric mean V_z/F (range: 171 to 258 L) of REC-3964 were comparable between single-dose levels. At a dose of 600 mg REC-3964, the peak and systemic exposure to REC-3964 was comparable between healthy elderly subjects and subjects aged ≤65 years.

Based on statistical analysis of dose proportionality using the power model across the 50 mg to 1200 mg single-dose range of REC-3964 in 30 subjects aged ≤65 years(Table S3), the systemic exposure of REC-3964 increased approximately dose proportionally as indicated by the slope estimates and lower bounds of the corresponding 90% CIs being slightly above 1 for AUC_{inf} (1.093; 1.012, 1.175) and AUC_{last} (1.092; 1.010, 1.174). The increase in C_{max} was less than dose proportional to the increase in REC-3964 dose, as indicated by the slope estimate being less than 1 (ie, 0.783) and the corresponding 90% CI not containing 1 (0.696, 0.869). The estimated GLSM ratios and corresponding 90% CI for dose-normalized PK parameters of REC-3964 in the ANOVA model indicated that the deviations from dose-proportionality increased with increasing single doses of REC-3964.

Based on the enantiomer ratios of REC-3974 to REC-3964, the peak and systemic exposure to the enantiomer REC-3974 was approximately 200× to 600× lower than for REC-3964, with geometric mean ER(C_{max}) and ER(AUC_{last}) values ranging from 0.0017 to 0.0060 across single-dose levels in healthy subjects aged ≤65 years. The geometric mean $T_{1/2}$ of REC-3974 ranged from 1.37 to 4.61 hours.

For elderly subjects aged >65 years, the peak and systemic exposures to the enantiomer REC-3974 were, respectively, 200× and 700× lower than for REC-3964, with geometric mean ER(C_{max}) and ER(AUC_{last}) of 0.0053 and 0.0014 at a dose of 600 mg REC-3964.

Table S2 Summary Statistics of Pharmacokinetic Parameters for REC-3964 in Plasma (Part A)

Parameter	Statistic	A1: 50 mg (N=6)	A2: 100 mg (N=6)	A3: 300 mg (N=6)	A4: 600 mg (N=6)	A5: 1200 mg (N=6)	A7: 600 mg (Elderly) (N=6)
AUC_{inf} (h·ng/mL)	n	6	6	6	6	6	5
	Geo Mean	2600	4970	16000	37800	81400	37500
	Geo CV%	37.7	17.4	27.6	28.2	45.0	52.0
	Min, Max	1540, 3780	4010, 6590	12100, 26900	28600, 60000	43200, 160000	16100, 57000
AUC_{last} (h·ng/mL)	n	6	6	6	6	6	6
	Geo Mean	2570	4910	15700	37500	80200	34800
	Geo CV%	37.6	17.1	28.2	28.1	45.8	49.0
	Min, Max	1530, 3740	3940, 6430	11900, 26700	28600, 59500	42100, 160000	15300, 55300
C_{max} (ng/mL)	n	6	6	6	6	6	6
	Geo Mean	878	1510	3700	6630	10100	7300
	Geo CV%	33.6	31.6	51.8	16.2	32.5	51.0
	Min, Max	554, 1230	973, 2030	1620, 6040	5030, 7940	6260, 16500	2920, 11100
T_{max} (h)	n	6	6	6	6	6	6
	Median	0.77	1.00	1.26	1.50	2.00	1.01
	Min, Max	0.52, 1.52	0.50, 2.02	1.00, 1.52	1.00, 4.00	1.00, 6.02	1.00, 1.52
$T_{1/2}$ (h)	n	6	6	6	6	6	5
	Geo Mean	7.66	6.93	9.52	7.63	8.02	9.71
	Geo CV%	31.7	39.8	34.1	36.4	28.6	41.4
	Min, Max	4.92, 10.7	4.08, 10.2	6.06, 14.9	5.24, 14.6	5.64, 11.1	5.03, 14.5
CL/F (L/h)	n	6	6	6	6	6	5
	Geo Mean	19.2	20.1	18.8	15.9	14.7	16.0
	Geo CV%	37.7	17.4	27.6	28.2	45.0	52.0
	Min, Max	13.2, 32.5	15.2, 25.0	11.2, 24.7	10.00, 21.0	7.48, 27.8	10.5, 37.2
V_z/F (L)	n	6	6	6	6	6	5
	Geo Mean	213	201	258	175	171	224
	Geo CV%	55.3	49.5	52.9	40.9	74.2	90.1
	Min, Max	112, 417	89.2, 342	112, 395	107, 356	60.9, 446	98.8, 776

CV%=coefficient of variation; geo=geometric; max=maximum; min=minimum; N=number of subjects in pharmacokinetic set; n=number of observations

Table S3 Statistical Analysis of the Dose Proportionality of REC-3964 – Power Model (Part A)

PK Parameter	Day	n	Intercept	Slope	90% CI of Slope
AUC_{inf} (h·ng/mL)	Day 1	30	3.522	1.093	(1.012, 1.175)
AUC_{last} (h·ng/mL)	Day 1	30	3.516	1.092	(1.010, 1.174)
C_{max} (ng/mL)	Day 1	30	3.729	0.783	(0.696, 0.869)

CI=confidence interval; n=number of observations; PK=pharmacokinetic

The dose proportionality analyses were performed using the power model: ln(PK) = intercept + slope*ln(dose) + ε, where "PK" is the PK parameter and "ε" is the error term. A value of slope=1 indicates dose proportionality. Cohort A7 (elderly subjects) was excluded from this analysis.

Plasma PK in Part B (Multiple Ascending Doses)

Across multiple-dose levels, the median T_{max} of REC-3964 was reached within ~1 hour on both Day 1 and Day 14 (Table S4). Like the single-dose PK of REC-3964, the geometric mean $T_{1/2}$ of REC-3964 on Day 14 ranged between 6.87 and 9.27 hours across the multiple-dose levels. Likewise, the geometric mean CL/F ranged from 13.1 to 16.4 L/h and the V_z/F from 161 to 179 L for REC-3964 on Day 14.

The mean plasma concentrations generally increased with increasing multiple dose levels. Steady-state concentrations of REC-3964 appeared to be reached by Day 3.

The highest C_{max} values of REC-3964 on Day 14 were observed for the 900 mg QD dose regimen (9470 ng/mL). For the 1.7-fold dose increase from 300 mg to 500 mg REC-3964 BID, the geometric mean C_{max} and AUC_{tau} of REC-3964 on Day 14 were, respectively, ~1.5- and ~1.6-fold higher for the 500 mg BID dose regimen (6370 ng/mL and 30400 h·ng/mL) than for the 300 mg BID dose regimen (4330 ng/mL and 19300 h·ng/mL).

The accumulation of REC-3964 on Day 14 compared to Day 1 ranged from 1.08 to 1.71 in terms of geometric mean AI(C_{max}) and from 1.52 to 1.93 in terms of AI(AUC_{tau}).

On Day 14, the geometric mean ER(C_{max}) and ER(AUC_{tau}) of REC-3974 to REC-3964 (ranging from 0.0021 to 0.0061) and geometric mean $T_{1/2}$ of REC-3974 (ranging from 3.05 to 3.64 hours) were within similar ranges as observed for the single-dose range of REC-3964. Due to complications with the quantification of REC-3974 in plasma, the enantiomer was not evaluable on Day 14 of the highest 900 mg QD dose level of REC-3964.

Table S4 Summary Statistics of Pharmacokinetic Parameters for REC-3964 in Plasma (Part B)

Day	Parameter	Statistic	B1: 100 mg TID (N=10)	B2: 300 mg BID (N=8)	B3: 500 mg BID (N=8)	B4: 900 mg QD (N=8)
1		n	10	8	8	8
	AUC_{tau} (h·ng/mL)	Geo Mean	3820	12800	16500	36800
		Geo CV%	37.1	32.1	52.9	69.6
		Min, Max	1970, 5530	8530, 22100	6440, 29700	9660, 74400
	C_{max} (ng/mL)	Geo Mean	1160	4010	4220	6590
		Geo CV%	63.4	28.9	45.3	66.0
		Min, Max	392, 2600	2740, 6770	2030, 6920	1620, 11100
	T_{max} (h)	Median	1.02	1.02	1.01	1.03
		Min, Max	0.50, 4.02	1.00, 2.00	0.52, 1.52	1.00, 2.00
14		n	8	8	8	8
	AUC_{tau} (h·ng/mL)	Geo Mean	7640	19300	30400	58500
		Geo CV%	30.7	36.9	51.2	44.0
		Min, Max	4010, 11400	13300, 37600	14900, 51000	36100, 135000
	AI AUC_{tau} (h·ng/mL)	Geo Mean	1.93	1.52	1.84	1.59
		Geo CV%	27.0	11.5	60.2	54.6
		Min, Max	1.50, 3.29	1.26, 1.75	0.811, 5.57	0.854, 3.73
	C_{max} (ng/mL)	Geo Mean	2020	4330	6370	9470
		Geo CV%	28.6	29.3	40.2	28.9
		Min, Max	1060, 2670	2620, 5870	3830, 11600	6430, 15800
	AI C_{max} (ng/mL)	Geo Mean	1.71	1.08	1.51	1.44
		Geo CV%	57.7	19.2	48.4	55.5
		Min, Max	0.846, 5.13	0.854, 1.33	0.612, 3.09	0.865, 4.23
	T_{max} (h)	Median	0.51	1.01	1.00	1.25
		Min, Max	0.40, 1.02	0.50, 1.53	0.50, 2.02	0.50, 3.00
	$T_{1/2}$ (h)	Geo Mean	9.27	7.99	6.87	7.24
		Geo CV%	28.0	15.8	27.9	26.1
		Min, Max	5.67, 13.3	6.63, 9.71	4.89, 10.4	4.87, 9.62
	CL/F (L/h)	Geo Mean	13.1	15.5	16.4	15.4
		Geo CV%	30.7	36.9	51.2	44.0
		Min, Max	8.78, 24.9	7.99, 22.5	9.80, 33.6	6.65, 24.9
	V_z/F (L)	Geo Mean	175	179	163	161
		Geo CV%	46.9	32.4	37.5	45.7
		Min, Max	97.4, 404	108, 232	91.7, 257	82.8, 270

AI=accumulation index (ie, ratio of Day 14/Day 1); BID=twice daily; CV%=coefficient of variation; geo=geometric; max=maximum; min=minimum; N=number of subjects in pharmacokinetic set; n=number of observations; QD=once daily; TID=three times daily

Urine PK in Part A (Single Ascending Doses)

After single doses of REC-3964 ranging from 50 mg to 1200 mg, the geometric mean $Ae_{0\text{-}t}$ of REC-3964 ranged from 1.89 to 69.8 mg, which was between 3.79% and 6.26% of the administered dose of REC-3964 (geometric mean Fe). The geometric mean renal clearance (CL_R) of REC-3964 ranged between 0.712 and 1.15 L/h (Table S5).

At a single dose of 600 mg REC-3964, the urinary excretion of REC-3964 was comparable between healthy elderly subjects and subjects aged ≤65 years, respectively, in terms of geometric mean $Ae_{0\text{-}t}$ (24.8 mg versus 37.6 mg), Fe (4.13% versus 6.26%), and CL_R (0.712 versus 1.00 L/h). For enantiomer REC-3974, the geometric mean $Ae_{0\text{-}t}$ values were very low, ranging from 0.0099 to 0.383 mg, with geometric mean CL_R values ranging from 1.08 to 2.52 L/h.

Table S5 Urine Pharmacokinetic Parameters of REC-3964 (Part A)

Parameter	Statistic	A1: 50 mg (N=6)	A2: 100 mg (N=6)	A3: 300 mg (N=6)	A4: 600 mg (N=6)	A5: 1200 mg (N=6)	A7: 600 mg (Elderly) (N=6)
	n	6	6	6	6	5	6
Ae_{0-t} (mg)	Geo Mean	1.89	5.66	15.0	37.6	69.8	24.8
	Geo CV%	27.3	22.0	31.5	48.5	42.5	31.6
	Min, Max	1.33, 2.66	4.03, 7.02	10.4, 22.4	22.2, 76.5	38.4, 107	13.7, 33.4
Fe (%)	Geo Mean	3.79	5.66	5.00	6.26	5.81	4.13
	Geo CV%	27.3	22.0	31.5	48.5	42.5	31.6
	Min, Max	2.66, 5.31	4.03, 7.02	3.45, 7.46	3.71, 12.7	3.20, 8.91	2.29, 5.57
CL_R (L/h)	Geo Mean	0.735	1.15	0.953	1.00	0.873	0.712
	Geo CV%	31.5	13.3	34.1	30.3	26.7	18.8
	Min, Max	0.425, 1.07	0.943, 1.35	0.687, 1.54	0.648, 1.42	0.571, 1.17	0.579, 0.895

CV%=coefficient of variation; geo=geometric; max=maximum; min=minimum; N=number of subjects in pharmacokinetic set; n=number of observations

Urine PK in Part B (Multiple Ascending Doses)

After administration of REC-3964 as 100 mg TID, 300 mg BID, 500 mg BID, or 900 mg QD dose regimens on Day 1, the geometric mean Ae_{tau} of REC-3964 ranged from 3.44 mg to 37.4 mg (geometric mean Fe range: 3.03% to 4.52%). On Day 14, the geometric mean Ae_{tau} of REC-3964 ranged from 8.11 to 56.9 mg (geometric mean Fe range: 5.65% to 8.11%) across multiple-dose levels. The geometric mean CL_R of REC-3964 ranged between 0.877 and 1.06 L/h on Days 1 and 14 (Table S6).

For the dose increase from 300 mg to 500 mg REC-3964 BID, respectively, the geometric mean Ae_{tau} of REC-3964 were 13.5 mg (Fe: 4.52%) and 15.2 mg (Fe: 3.03%) on Day 1 and increased from 17.0 mg (5.65%) to 31.7 mg (6.35%) on Day 14.

For enantiomer REC-3974, the geometric mean Ae_{tau} values ranged from 0.0221 to 0.204 mg on Day 1, and from 0.0503 to 0.315 mg on Day 14. The geometric mean CL_R of REC-3974 ranged between 1.26 and 2.73 L/h on Days 1 and 14.

Table S6 Urine Pharmacokinetic Parameters of REC-3964 (Part B)

Day	Parameter	Statistic	B1: 100 mg TID (N=10)	B2: 300 mg BID (N=8)	B3: 500 mg BID (N=8)	B4: 900 mg QD (N=8)
1		n	10	8	8	8
	Ae_{tau} (mg)	Geo Mean	3.44	13.5	15.2	37.4
		Geo CV%	41.1	34.4	95.7	101
		Min, Max	1.72, 6.17	9.16, 27.0	2.41, 32.6	6.20, 100.0
	Fe (%)	Geo Mean	3.44	4.52	3.03	4.16
		Geo CV%	41.1	34.4	95.7	101
		Min, Max	1.72, 6.17	3.05, 9.00	0.481, 6.52	0.689, 11.1
	CL_R (L/h)	Geo Mean	0.900	1.06	0.917	1.02
		Geo CV%	23.8	26.0	39.4	30.7
		Min, Max	0.562, 1.28	0.746, 1.52	0.374, 1.21	0.642, 1.55
14		n	8	8	8	8
	Ae_{tau} (mg)	Geo Mean	8.11	17.0	31.7	56.9
		Geo CV%	45.5	58.1	52.6	48.2
		Min, Max	4.05, 13.4	7.29, 45.4	15.2, 57.4	28.6, 106
	Fe (%)	Geo Mean	8.11	5.65	6.35	6.32
		Geo CV%	45.5	58.1	52.6	48.2
		Min, Max	4.05, 13.4	2.43, 15.1	3.05, 11.5	3.18, 11.8
	CL_R (L/h)	Geo Mean	1.06	0.877	1.04	0.971
		Geo CV%	30.6	33.4	22.0	26.0
		Min, Max	0.733, 1.73	0.508, 1.39	0.645, 1.32	0.782, 1.51

BID=twice daily; CV%=coefficient of variation; geo=geometric; max=maximum; min=minimum; N=number of subjects in pharmacokinetic set; n=number of observations; QD=once daily; TID=three times daily

Exploratory (Part B Only)

The mean 4β-OH-cholesterol levels on Day 14 were comparable across multiple-dose levels of REC-3964, ranging from 26.0 to 31.3 ng/mL. The minimal increases on Day 14 compared to baseline at predose on Day 1 (difference between Day 14 and baseline ranging from 0.2 to 3.1 ng/mL) were not indicative of CYP3A-mediated changes in 4β-OH-cholesterol levels at any of the tested multiple-dose levels.

Conclusions

Safety

- The administration of single oral doses of REC-3964 was well tolerated by healthy subjects at doses from 50 mg to 1200 mg, including a 600 mg dose of REC-3964 administered to healthy elderly subjects aged >65 years. Also, multiple oral doses of REC-3964 were well tolerated by healthy subjects at total daily doses from 300 mg to 1000 mg for 14 days (in dose regimens of 100 mg TID, 300 mg BID, 500 mg BID, or 900 mg QD).
- No deaths, SAEs, or discontinuations due to AEs were reported during the study. Also, no clinically relevant safety laboratory, 12-lead ECG, telemetry monitoring, vital signs, or physical examination findings were reported.

Pharmacokinetics

Part A

- After single oral doses of REC-3964, the median T_{max} of REC-3964 in plasma ranged from 0.77 to 2.00 hours and the geometric mean $T_{1/2}$ from 6.93 to 9.71 hours, independent from age group (≤65 years or >65 years). Likewise, the geometric mean CL/F (range: 14.7 to 20.1 L/h) and V_z/F (range: 171 to 258 L) were comparable across the 50 mg to 1200 mg dose range and age groups.

- Across the 50 mg to 1200 mg single-dose range of REC-3964 in subjects aged ≤65 years, the 90% CIs of the slope estimates did not contain 1 for any plasma PK parameter. However, the systemic exposure to REC-3964 increased approximately dose proportionally as indicated by slopes and lower bounds of the 90% CIs being just above 1 for AUC_{inf} (1.093; 90% CI: 1.012, 1.175) and AUC_{last} (1.092; 90% CI: 1.010, 1.174), while the peak exposure (C_{max}) increased less than dose proportionally (0.783; 90% CI: 0.696, 0.869).
- At a dose of 600 mg REC-3964, the peak and systemic exposure to REC-3964 was comparable between subjects aged ≤65 years and elderly subjects aged >65 years.
- The enantiomer ratios for the peak and systemic plasma exposure of REC-3974 compared to REC-3964 ranged from 0.0017 to 0.0060 across the single-dose range in subjects aged ≤65 years and from 0.0014 to 0.0053 for subjects aged >65 years.
- The urinary excretion was low for REC-3964, with geometric mean $Ae_{0\text{-}t}$ ranging from 1.89 to 69.8 mg (Fe: 3.79% to 6.26%; CL_R: 0.712 to 1.15 L/h), and minimal for REC-3974 ($Ae_{0\text{-}t}$: ≤0.383 mg) across the single-dose range and age groups.

Part B

- After multiple oral doses of 100 mg TID, 300 mg BID, 500 mg BID, or 900 mg QD REC-3964 for 14 days, the median T_{max} of REC-3964 was reached within ~1 hour on Days 1 and 14. On Day 14, the geometric mean $T_{1/2}$ ranged from 6.87 to 9.27 hours.
- Steady-state concentrations of REC-3964 appeared to be reached by Day 3. The accumulation of REC-3964 in terms of geometric mean AI(C_{max}) and AI(AUC_{tau}) ranged between 1.08 and 1.93 on Day 14.
- After multiple oral doses of 100 mg TID, 300 mg BID, and 500 mg BID REC-3964 for 14 days, the enantiomer ratios ranged from 0.0021 to 0.0061.
- On Day 14, the geometric mean Ae_{tau} ranged from 8.11 to 56.9 mg (Fe: 5.65% to 8.11%; CL_R: 0.877 to 1.06 L/h) for REC-3964 and ≤0.315 mg of REC-3974 was excreted in urine across the tested multiple-dose range.

<u>Exploratory</u>

- After administration of multiple oral doses of REC-3964 at total daily doses from 300 mg to 1000 mg, the mean 4β-OH-cholesterol levels on Day 14 were not suggestive of changes in CYP3A activity at any of the tested multiple-dose levels.