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Research Article

Safety, Tolerability and Pharmacokinetics of a Novel Triaminopyrimidine ZY-19489: A Randomised Double-blind Placebo-control Phase-1 Study among Healthy Indian Participants

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I N F O

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A B S T R A C T

Background: ZY-19489 is a triaminopyrimidine comprising the novel antimalarial class.

Methods: This was a phase-1, two-part double-blind, placebo-controlled study conducted in Ahmedabad, India. Part-1 was a single-dose single-cohort study. Part-2 was a multiple-ascending-dose study. Healthy participants aged 18-55 years were included. The primary objective was to evaluate the safety and tolerability of ZY-19489 in healthy adult Indian participants. This study was registered with CTRI/2021/08/035449 and on clinicaltrials.gov (NCT05206201).

Findings: Eight participants were enrolled in part-1 (450 mg) and 16 participants were enrolled in part-2, a multiple ascending dose part, (two cohorts; 300 and 500 mg once daily for three days). All participants were randomised to a 3:1 ratio (ZY-19489:placebo) within the cohort. The majority of adverse events (AEs) reported were mild in severity. One of six participants in part-1 and one of twelve participants in part-2, exposed to ZY-19489, experienced grade-2 AEs (enteric fever, dengue, and leg pain). One of six participants in part-1 and one of twelve participants in part-2, exposed to ZY-19489, experienced grade-3 AEs (GGT increase and epigastric pain). Following single-dose administration, ZY-19489 displayed slow oral absorption with a median T_{max} of 7.5 h and a mean elimination half-life of 90 h. Following multiple dose administration of ZY-19489 for three consecutive days, on day 3, the maximum plasma concentration was achieved at 6.25 h (median T_{max}) after dosing.

Conclusion: ZY-19489 was well-tolerated up to 500 mg once a day for three days. It displayed good oral absorption, dose-proportional increase in exposure, and long elimination half-life.

Keywords: ZY19489, Safety, Pharmacokinetic, Healthy Participants, Antimalarial

Introduction

Malaria remains a grave concern for global health with an estimated 247 million cases and 619000 deaths in 2021.[1] There are reports of artemisinin-resistant *Plasmodium falciparum* cases in the Greater Mekong sub-region. Malaria control and elimination programmes are at risk due to the emergence and spread of such resistance.[2] There is a need of novel antimalarial treatments to reduce disease burden and death.

ZY-19489 (also known as AZ-13721412 and MMV-674253) is a triaminopyrimidine antimalarial drug candidate that has been developed by Zydus Lifesciences Ltd. *In vitro* and *in vivo* models of malaria suggest that ZY-19489 and its major active metabolite ZY-20486 are highly potent towards the asexual blood-stage of *P. falciparum*.[3] ZY-19489 was found safe and was well tolerated by healthy study participants till 1500 mg single dose during the first-in-human study conducted in Australia. Rapid clearance of parasitaemia was observed in all participants after ZY-19489 administration. PK/ PD (Pharmacokinetics/ pharmacodynamics) models reported a parasite clearance half-life ($PCt_{1/2}$) of 6.6 h to 7.1 h. These results supported further clinical development of ZY-19489 as an antimalarial drug.[4]

The present report describes a two-part phase-1 clinical study aimed to evaluate the safety, tolerability, and pharmacokinetics of ZY-19489 in healthy Indian volunteers. The first part was a single-dose bridging PK study with orally administered ZY-19489 (450 mg) or matching placebo with 3:1 randomisation to compare pharmacokinetic properties among the study populations from different geographical regions. Part-2 was a multiple ascending doses (MAD) study (300 mg cohort-1, 500 mg cohort-2) of three divided daily doses of ZY-19489 or matching placebo administered orally with 3:1 randomisation. Collectively, this study aimed to determine whether ZY-19489 represents a viable antimalarial drug candidate for continued clinical development.

Materials and Methods

This clinical trial was conducted at Zydus Research Centre (Ahmedabad, India) after getting approval from the Sangini Hospital Ethics Committee (Ahmedabad, India) and the Central Drugs Standard Control Organization of India. Written informed consent was taken from all participants before performing any study-related procedure. This study was registered with the clinical trial registry of India (CTRI/2021/08/035449) and on clinicaltrials.gov (NCT05206201). This trial was conducted in accordance with the applicable local regulations. The first subject was enrolled on August 25, 2021 and the study was completed (last subject's last visit) on January 21, 2022.

Study Design

This study was a randomised, double-blind, placebo-controlled study with two parts. Part-1 was a single-dose single-cohort study. Eight participants were enrolled in this part. Six participants received ZY-19489 (450 mg) and two received a matching placebo. Part-2 was a multiple-ascending-dose study with once daily for three-day dosing. It was conducted in two cohorts (cohort-1: 300 mg, cohort-2: 500 mg). Eight participants were enrolled in each cohort. Six participants received ZY-19489 and two received a matching placebo once a day for three days in each cohort.

Study Participants

Healthy male and female (non-pregnant, non-lactating) participants with ages between 18 and 55 years, a minimum weight of 50 kg and BMIs ranging from 18.5 to 30.0 kg/ m^2 (both inclusive) were eligible for inclusion in this study. The detailed inclusion and exclusion criteria have been described in Table S1 (provided in the Appendix). A total of 102 participants were screened and 24 participants were enrolled in the study.

Procedure

Eligible healthy participants were enrolled for a 28-day screening period to ensure that they met all inclusion criteria and none of the exclusion criteria. Participants were admitted to the clinical research unit on the day (-1) and were discharged after 72 hours post-dose (for part-2 post third dose). Then they reported to the clinical facility as outpatient visits till the end of the study (EOS) visit; day 28 for part-1 and day 30 for part-2. Out of eight enrolled participants of each cohort, six received ZY-19489 (API in capsule) and two received a placebo with 240 mL water after fasting for at least 10 h; no food was allowed until 4 h after dosing. A sentinel dosing strategy was employed for this study. Two sentinel participants (one randomised to ZY-19489 and the other randomised to placebo) were dosed first. After a review of the blinded safety data of up to 48 hours post-dosing by the investigator, the remaining six participants were enrolled in each cohort.

In part-1, six participants received a single dose of ZY-19489 (450 mg) and two participants received a placebo. The safety data review team (SDRT) reviewed blinded safety and pharmacokinetics (PK) data till day 14 to decide on the commencement of part-2. SDRT was comprised of medical experts and PK experts from Medicine for Malaria Venture (MMV), and Principal Investigator and sponsored medical experts from Zydus Lifesciences Ltd.

Part-2 was conducted in two cohorts (cohort-1: 300 mg, cohort-2: 500 mg). Each cohort consisted of eight participants. All participants received either ZY19489 or a

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placebo once a day for three days as per 3:1 randomisation. The SDRT reviewed blinded safety and PK data till day 16 before the commencement of the next cohort.

Randomisation

Participants were randomised to either ZY-19489 or placebo in a 3:1 ratio. As it was a double-blind study, the investigator and participants were blinded to the treatment starting from the randomisation until the database lock. Placebo was identical to the active treatment ZY-19489 in appearance and packaging. Sentinel participants were initially randomised to a 1:1 ratio and the remaining six participants were randomised to a 5:1 ratio. SAS software version 9.4 was used to generate randomisation. The investigator was provided with an emergency un-blinding code to be used in case of emergency; however, no such incident occurred that required emergency un-blinding.

Blood Sampling for Pharmacokinetics

In part-1, a total of 26 venous blood samples were collected at pre-dose, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 12, 16, 24, 36, 48, and 72 hours post-dose, on day 7, day 14, day 21 and day 28 for pharmacokinetic analysis. In part-2, a total of 30 venous blood samples were collected at pre-dose, and 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours post-IMP administration on day 0 and day 2, at pre-dose and 6 hours post-dose on day 1, at day 3, day 5, day 9, day 16, day 23 and day 30. Venous blood samples (6 mL) were collected in a K_2-EDTA vacutainer for the evaluation of pharmacokinetic parameters. Plasma was separated from the blood samples and was stored frozen at -70 ± 20 °C until analysis.

Urine Sampling for Pharmacokinetics

In part-1, urine samples were collected at pre-dose and at defined time intervals till 72 hours post-dosing. A pooled urine sample was collected from the total collected urine at each time interval and two aliquots (around 4.5 mL each) of the samples were stored frozen at -70 ± 20 °C until analysis.

Safety Assessments

In both parts, safety was assessed by the incidence, severity, and relationship of adverse events (AEs), clinical examination, frequent assessments of vital signs, continuous ECG monitoring, triplicate 12-lead ECGs, continuous ECG recording (Holter recording) with the evaluation of ECG parameters and clinical laboratory investigations (haematology, biochemistry, and urinalysis).

In part-1, a physical examination was conducted at the time of screening, check-in, day 1, day 3, day 7, day 14, day 21 and day 28. Vital signs were measured during subject screening, check-in, pre-dose, 1, 2, 4, 6, 12, 24, 36, 48 and 72 hours post-dose, on day 7, day 14, day 21 and day 28. Electrocardiograms were recorded during subject screening, check-in, pre-dose, 1, 2, 4, 6, 8, 12, 24, 48 and 72 hours post-dose, on day 7, day 14, day 21 and day 28.

In part-2, a physical examination was conducted at the time of screening, check-in, day 1, day 3, day 5, day 9, day 16, day 23 and day 30. Vital signs were measured during subject screening, check-in, on day 0 to day 2 at pre-dose, 1, 2, 4, 6, and 12 hours post-dose, 24, 36, 48 and 72 hours after the last dose, and on day 9, day 16, day 23 and day 30. Electrocardiograms were recorded during subject screening, check-in, on day 0 to day 2 at pre-dose, 1, 2, 4, 6, 8 and 12 hours post-dose and 24, 48 and 72 hours after the last dose, and on day 9, day 16, day 23 and day 30.

Study Sample Bioanalysis

The plasma and urine samples were analysed for the determination of ZY-19489 and its active metabolite ZY-20486. The quantitative analysis of analyte(s) in plasma and urine samples was performed using separately validated high-performance liquid chromatography with tandem mass spectrometry (LC-MS/ MS) assays. The assay validation was carried out in accordance with the FDA guideline for Bioanalytical Method Validation (May 2018). A gradient elution or isocratic chromatographic separation of analyte(s) and internal standard was achieved on a Zorbex extended C18 100 x 4.6 mm analytical column. The plasma samples were purified using liquid-liquid extraction and urine samples were purified with solid phase extraction (SPE) techniques. The purified samples were analysed on API4000 or API3200 mass detector with an electrospray turbo ion source (ESI) operated in positive mode. The mass transition used for the quantitative determination was m/z 466.2 to 395.3, m/z 452.3 to 286.2 and m/z 426.2 to 355.2 for ZY-19489, ZY-20486, and internal standard respectively. The linearity ranges for plasma were 1 to 500 ng/mL for ZY-19489 and 2 to 200 ng/mL for ZY-20486. The linearity range for urine was 3 to 600 ng/mL for both ZY-19489 and ZY-20486. Precision and accuracy of the lower limit of quantification (LLOQ) and other quality control samples were found to be within 20% and 15% respectively. Both the assays were selective, sensitive, devoid of any carryover, and suitable for the clinical sample analyses. The study samples were analysed along with calibration curve standard and quality control samples distributed throughout each analytical run. The chromatographic peaks were processed using analyst software 1.6.2. A linear regression model with a $1/x^2$ weighing factor was used to process the study samples. The concentration of analyte(s) of each study sample from the analytical run was reported based on predefined run acceptance criteria.

Statistical Analysis

Considering general phase-1 study requirements, it was determined that the targeted sample size of 8 participants

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per cohort was appropriate to evaluate the primary safety outcome. The primary safety objective was evaluated by descriptive summary statistics for the laboratory parameters, i.e., haematology, serum biochemistry, urine analysis, AEs, physical examinations, and vital signs (blood pressure, pulse, and body temperature) for each cohort. Non-compartmental pharmacokinetic parameters were calculated using Phoenix® WinNonlin software version 8.3. Data sets were derived based on source data. Listing of participant data, tabulation of descriptive statistics, and statistical analysis were performed primarily using SAS® (version 9.4; SAS Institute Inc., USA). Descriptive statistics (mean, median, standard deviation, minimum, maximum, geometric mean, and coefficient of variation) were used for pharmacokinetic analysis.

Results

Participants

Eight participants were enrolled in part-1 and sixteen participants were enrolled in part-2 (eight participants in each cohort) of the study (Figure 1). Out of eight participants in each cohort, six were randomised to ZY19489 and two to placebo. In total, 18 out of 24 participants received ZY19489 and 6 out of 24 received a placebo. Nine out of 24 participants (37.5%) were female and 15 (62.5%) were male. One participant (female) in part-2 cohort-2 (500 mg dose cohort) was removed from the study due to an adverse event (epigastric pain) on the day of the first dosing. Demographic characteristics of the participants included in the safety population of ZY-19489-dosed participants and placebo participants were similar. Demographic details for each treatment group have been provided in Table 1.

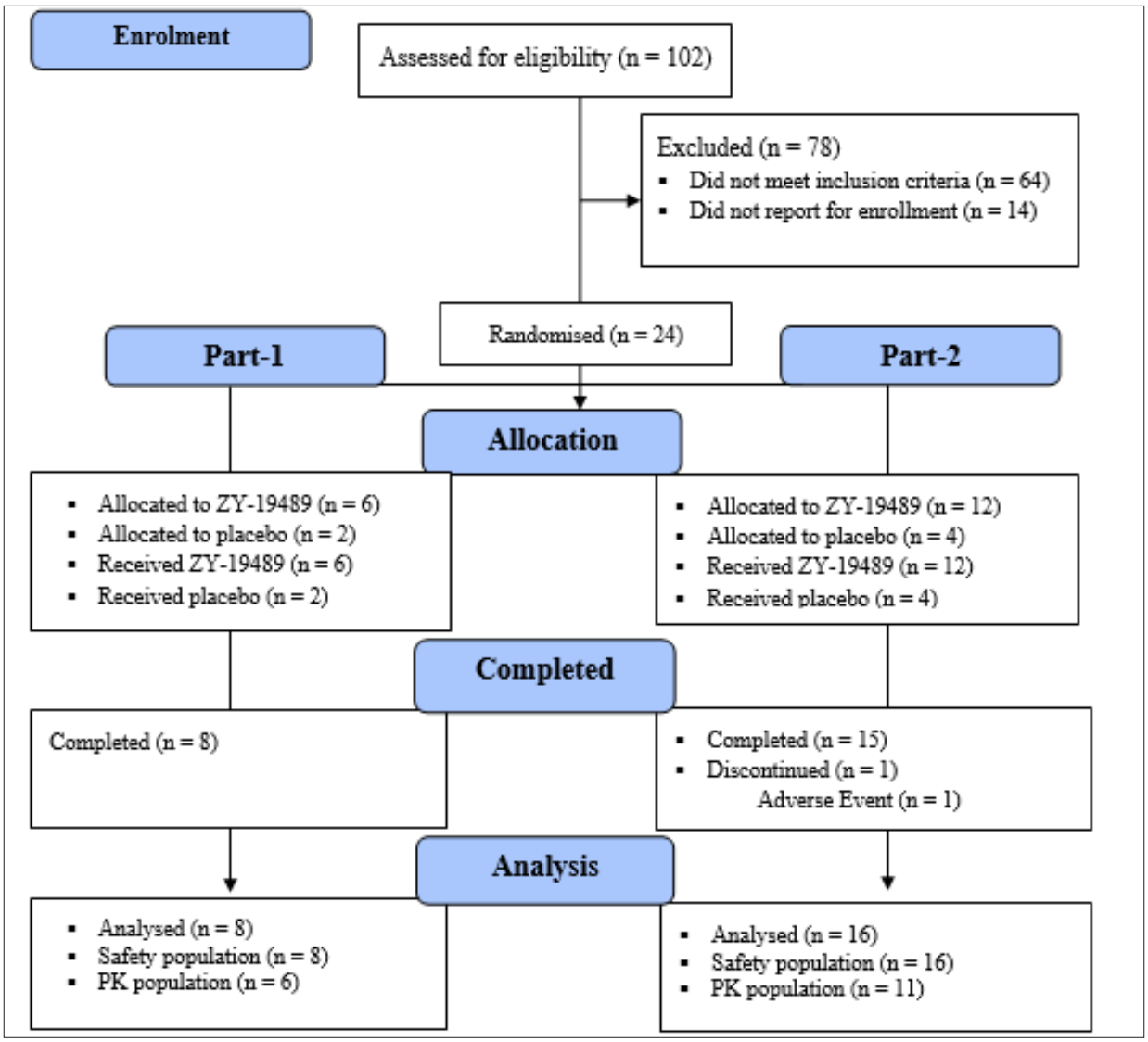

Figure 1.Disposition of Participants

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Table 1.Demographic Characteristics

Treatment Group		Age		Gender n (%)		Weight (kg)	Height (cm)	BMI (kg/m²)	
		Mean ± SD	Range	Male	Female	Mean ± SD	Mean ± SD	Mean ± SD	Range
Part-1	ZY19489 450 mg (N = 6)	40.2 ± 6.3	32-48	4 (66.7)	2 (33.3)	61.43 ± 6.10	160.67 ± 6.86	23.9 ± 3.6	20.19-29.67
	Placebo (N = 2)	36.0 ± 12.7	27-45	1 (50.0)	1 (50.0)	54.65 ± 3.04	158.50 ± 14.85	21.9 ± 2.9	19.88-23.96
	Overall (N = 8)	39.1 ± 7.4	27-48	5 (62.5)	3 (37.5)	59.74 ± 6.14	160.13 ± 8.13	23.4 ± 3.3	19.88-29.67
Part-2	ZY19489 300 mg (N = 6)	38.7 ± 9.1	25-49	4 (66.7)	2 (33.3)	62.82 ± 8.46	165.50 ± 2.95	22.9 ± 2.7	19.66-26.47
	ZY19489 500 mg (N = 6)	37.0 ± 4.8	32-45	3 (50.0)	3 (50.0)	67.53 ± 8.86	158.67 ± 8.64	26.7 ± 1.5	24.68-28.07
	Placebo (N = 4)	35.0 ± 5.5	27-39	3 (75.0)	1 (25.0)	62.60 ± 5.47	163.00 ± 8.04	23.5 ± 0.7	22.68-24.30
	Overall (N = 16)	37.1 ± 6.6	25-49	10 (62.5)	6 (37.5)	64.53 ± 7.86	162.31 ± 7.09	24.5 ± 2.5	19.66-28.07

SD: Standard Deviation, BMI: Body Mass Index, N: Number of subjects in a specified treatment; n: Number of subjects having non-missing values.

Safety and Tolerability

In part-1, a total of 5 out of 6 participants (83.33%) who received ZY-19489 and none of the placebo-dosed participants experienced at least one AE; all AEs were resolved by the end of the study. Among the six participants dosed with ZY-19489, for two (33.33%), the AEs were considered by the investigator to be related to the study investigational medicinal product, and for 3 (50.00%), the AEs were considered to be not related to the study investigational medicinal product. The majority of participants reported grade 1 (66.67%) AEs. One out of six ZY-19489 dosed participants (16.67%) experienced grade 2 AEs (enteric fever and dengue infection) which was marked as not related to the study investigational medicinal product. One grade-3 AE (gamma-glutamyl transferase increase) was reported on day 14 of ZY-19489 administration which was resolved without any intervention. There were no clinically significant abnormal vital signs, haematology, or 12-lead ECG parameters. None of the participants reported QTcF > 480 msec or a significant increase in QTcF (i.e., > 30 msec) compared to baseline.

In part-2, a total of 7 out of 12 participants (58.33%) dosed with ZY-19489 and 2 out of 4 placebo-dosed participants (50.00%) experienced at least one AE; all AEs were resolved by the end of the study. Six out of 12 participants (50.00%) dosed with ZY-19489 and 2 out of 4 placebo-dosed participants (50.00%) experienced a total of 15 grade 1 AEs. One out of 12 participants (8.33%) dosed with ZY-19489 experienced a grade 2 (leg pain) AE. One out of 12 participants dosed with ZY-19489 experienced grade-3 AE (epigastric pain) which was reported on day 0 around 3 hours after the first dose. Myocardial infarction was ruled out with an evaluation of ECG and cardiac marker (troponin I). The participant was withdrawn from the study and kept under observation till check out and safety assessment was continued till the end of the study. The most frequent AEs were gastrointestinal disorders and investigations-related AEs. Four out of 12 participants (33.33%) dosed with ZY-19489 reported increased liver enzyme (either ALT increase or both ALT and AST increase), and 1 out of 4 participants (25%) in the placebo treatment reported an ALT increase. Mild ALT/ AST increases (maximum up to 2.5 x ULN) were observed around 2-3 days after the last dose administration and were resolved within 3-5 days of onset. All the participants with AEs of AST/ ALT increase were asymptomatic. None of the participants reported ALT/ AST increase ≥ 3 x ULN. None of these participants reported bilirubin levels higher than the upper normal limit. One out of 12 participants (8.33%) dosed with ZY-19489 and 1 out of 4 participants (25%) in the placebo treatment reported a decrease in haemoglobin. All investigations-related AEs were mild in severity and were resolved without intervention or sequelae (details are mentioned in Tables S2 and S3 provided in the Appendix).

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Effect on QTcF assessed by Continuous Holter Monitor

Categorical, morphological, central tendency, and concentration-response analyses were performed from extracted ECGs matched with plasma concentration time points. During categorical analysis, the most frequently reported abnormal value was a relative increase from baseline in heart rate (> 25%). This increase occurred in both the ZY-19489 dose group as well as in the placebo group without any obvious difference between ZY-19489 and placebo. During morphological analysis, no effect of the treatment with ZY-19489 could be discerned on ECG morphology when compared to the placebo. The central tendency analysis of QTcF interval indicated that in both the treatments (placebo and ZY-19489 dose groups), QTcF decreased, reaching a maximum typically between 6 and 10 h after dose administration. The concentration-response analysis indicated that the maximum predicted increase (90% CI) in ΔΔQTcF was 5.81 (min to max: 1.34 to 10.3) msec at the dose of 500 mg of ZY-19489 after the last dose administration on day 2.

Pharmacokinetics

After single-dose administration, ZY-19489 showed a slower rate of oral absorption with the median T_{max} (min-max) around 7.5 hours (5.0-10.0 hours) and a longer mean elimination half-life (SD) of 90.33 (29.06) hours. A negligible (2.4%) amount of unchanged ZY-19489 was recovered in the urine suggesting that renal is a minor route of elimination. For population PK assessment, the pharmacokinetics data of ZY-19489 generated in healthy Indian participants were compared with the data generated in healthy Australian participants.[4] The PK parameters C_{max}, $t_{1/2}$, and AUC determined in Indian participants showed marginally lower values, but the differences were statistically non-significant ($p > 0.05$), whereas the T_{max} value was on the higher side ($p = 0.01$).

The pharmacokinetic parameters of ZY-19489 and its active metabolite ZY-20486 determined after single dosing have been presented in Table 2. A comparison of the pharmacokinetic parameters of Indian and Australian participants has been shown in Table 3. The concentration-time profiles of ZY-19489 (mean ± standard deviation of the mean) after single oral administration of 450 mg has been shown in Figure 2.

The mean plasma concentration-time plot of 450 mg dose has been demonstrated in Figure S1 (provided in the Appendix).

Table 2.Pharmacokinetic Parameters of ZY-19489 and ZY-20486 at 450 mg Single Dose

PK Parameters	ZY-19489 (N = 6[a])	ZY-20486 (N = 6)
T_{max} (hr)**	7.5 (5.0-10.0)	9.250 (5.0-48.0)
C_{max} (ng/mL)	302.9 ± 66.1	17.2 ± 8.9
AUC_{0-t} (hr*ng/mL)	24186.9 ± 7375.5	2532.1 ± 1436.6
AUC_{0-inf} (hr*ng/mL)	24610.3 ± 7448.2	3408.7 ± 1445.5
$T_{1/2}$ (hr)	90.3 ± 29.1	177.2 ± 115.0

N: Number of subjects; T_{max}: Time to reach maximum plasma concentration; C_{max}: Maximum plasma concentration; AUC_{0-t}: Area under the plasma concentration-time curve from time 0.0 h to the last measurable time point; AUC_{0-inf}: Area under the plasma concentration time curve extrapolated to infinity; $T_{1/2}$: Elimination half-life; ng: Nanogram; hr: Hours; mL: Millilitre.
All data have been presented here as mean ± SD (Standard Deviation) except T_{max}. **Median values for T_{max} have been reported with minimum and maximum observation.

Table 3.Comparison of PK Parameters of ZY-19489 between Indian and Australian populations at 450 mg Single Dose

PK Parameters	Australian Population Mean-SE (N = 6)	Indian Population Mean-SE (N = 6)	p Value
T_{max} (hr)**	5.2-0.5	7.6-0.5	0.0105
C_{max} (ng/mL)	460.9-52.4	302.9-52.4	0.0588
AUC_{0-t} (hr*ng/mL)	25866.2-3900.9	24186.9-3900.9	0.7671
AUC_{0-inf} (hr*ng/mL)	26497.8-4074.8	24610.3-4074.8	0.7500
$T_{1/2}$ (hr)	110.2-18.4	90.3-18.4	0.4613

N: Number of subjects; T_{max}: Time to reach maximum plasma concentration; C_{max}: Maximum plasma concentration; AUC_{0-t}: Area under the plasma concentration-time curve from time 0.0 h to the last measurable time point; AUC_{0-inf}: Area under the plasma concentration time curve extrapolated to infinity; $T_{1/2}$: Elimination half-life; SE: Standard error; ng: Nanogram; hr: Hours; mL: Millilitre.

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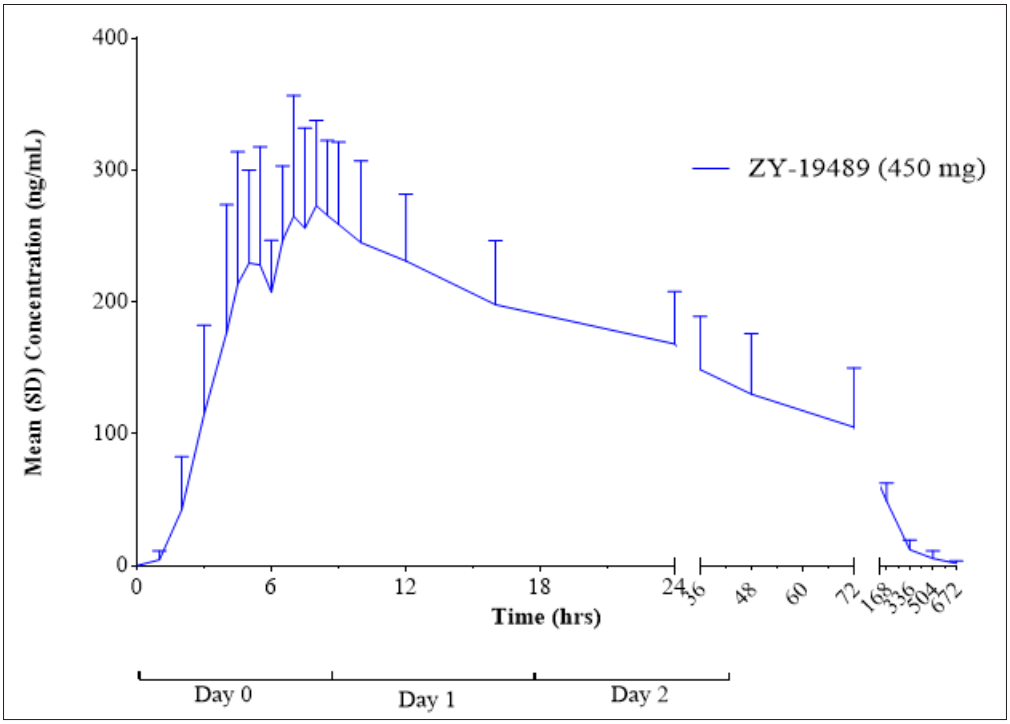

Figure 2.Concentration-Time Plot (Mean ± Standard Error) of ZY-19489 after Single Oral Administration of 450 mg Doses to Healthy Human Participants

In part-2, ZY-19489 PK parameters showed a dose-proportional increase in the AUC and C_{max}. The T_{max} was achieved approximately at 5.8 to 6.5 hours post-last dose. Racc AUC_{0-24} was about 3.6 to 3.8 and 5.4 to 5.7 for ZY-19489 and its active metabolite ZY-20486 respectively.

The PK parameters of ZY-19489 after a multiple-dose administration study have been presented in Table 4. The mean plasma concentration-time plot of 300 and 500 mg doses has been demonstrated in Figure 3.

The mean plasma concentration-time plot of 300 and 500 mg doses has been demonstrated in Figure S2 (provided in the Appendix).

Table 4.PK Parameters of ZY-19489 and ZY-20486 by Dose Group - Part-2

PK Parameters	ZY-19489		ZY-20486	
	300 mg (N = 6)	500 mg (N = 5)	300 mg (N = 6)	500 mg (N = 5)
T_{max} (hr)** (After first dose)	54.5 (52.0-58.0)	54.0 (53.0-55.0)	56.0 (53.0-72.0),	55.0 (54.0-120.0)
T_{max} (hr)** (After third dose)	6.5 (4.0-10.0)	5.8 (5.0-7.0)	8.0 (5.0-24.0)	5.8 (6.0-72.0)
C_{max} (ng/mL)	440.9 ± 226.0	1188.7 ± 622.1	37.0 ± 31.4	87.4 ± 54.7
AUC_{0-t} (hr*ng/mL)	50854.4 ± 19500.2	120524.2 ± 61013.2	7705.5 ± 6271.2	22920.6 ± 17045.9
AUC_{0-inf} (hr*ng/mL)	51539.5 ± 19722.6	122409.6 ± 61586.1	8314.2 ± 6163.6	23770.3 ± 17337.5
Racc AUC_{0-24}	3.6 ± 0.7	3.8 ± 0.6	5.7 ± 1.4	5.5 ± 0.9

N: Number of subjects; T_{max}: Time to reach maximum plasma concentration; C_{max}: Maximum plasma concentration; AUC_{0-t}: Area under the plasma concentration-time curve from time 0.0 h to the last measurable time point; AUC_{0-inf}: Area under the plasma concentration time curve extrapolated to infinity; Racc: Drug accumulation ratio; ng: Nanogram; hr: Hours; mL: Millilitre

All data have been presented here as mean ± SD (Standard Deviation) except T_{max}.

**Median values for T_{max} have been reported with minimum and maximum observation.

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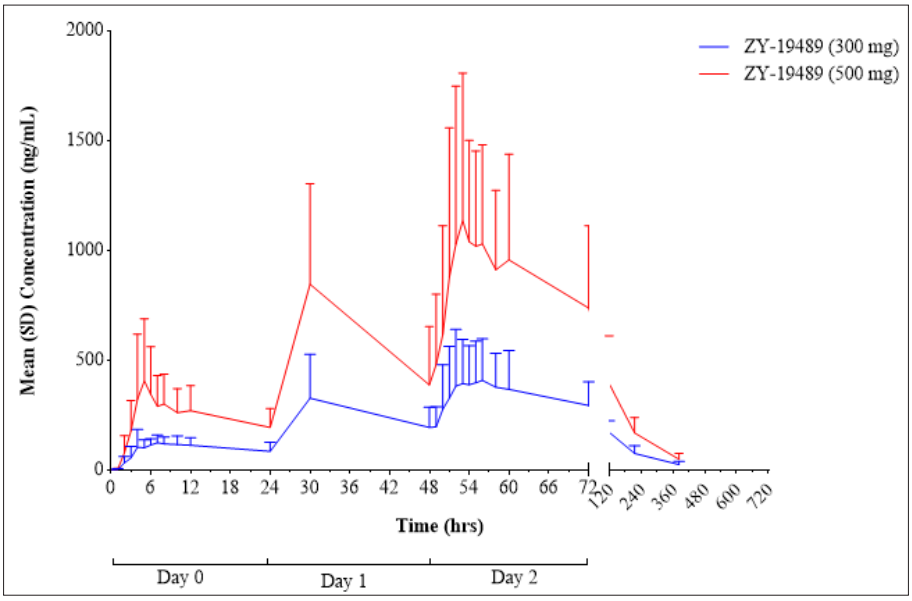

Figure 3.Concentration-Time Plot (Mean ± Standard Error) of ZY-19489 after Administration of 300 mg and 500 mg Once A Day for Three Days to Healthy Human Participants

Discussion

This phase-1 study was conducted to investigate the safety, tolerability, and pharmacokinetics of ZY-19489 in healthy Indian adult human participants. The PK data have shown the promising profile of ZY-19489 after single as well as multiple-dose regimens and are well supported for further clinical development. Safety results indicated that ZY-19489 is safe and well tolerated up to 500 mg once a day for three days. At a higher dose (500 mg dose cohort), the incidence of adverse events was higher compared to a lower dose (300 mg dose cohort) or placebo. The majority of the Adverse Events (AEs) considered related to ZY-19489 were mild and were resolved without sequelae. In each part, one grade 3 AE was reported. ZY-19489 systemic concentration was more than the estimated median MPC90 (39.3 ng/mL) for more than 7 days, along with a long elimination half-life, which supports its further development as an antimalarial drug candidate. Female participants generally showed higher C_{max} and AUC compared to male participants however, the participants enrolled were limited and not powered enough to demonstrate the statistically meaningful differences between genders in this study.

The increasing trend of exposure (C_{max} and AUC) following multiple dosing once daily for three days indicates drug accumulation. This was confirmed by the accumulation index ratio of AUC ($RaccAUC_{0-24}$) of 3.6-3.8 and it is well supported by the long elimination half-life (90 h). Similarly, its active metabolite showed a marginally higher accumulation index ratio of AUC ($RaccAUC_{0-24}$) of 5.6 to 5.7 and was very well in agreement with a relatively long elimination half-life (177 h) than that of the parent, ZY-19489.

The first-in-human study conducted in Australia had shown good safety, favourable pharmacokinetics, and potential antimalarial activity of ZY-19489.[4] While comparing the pharmacokinetic data, no statistically significant difference in PK parameters for ZY-19489 between Indian and Australian populations was observed except for T_{max} (p value < 0.05).

Limitations

Although this phase-1 study provides valuable information on ZY-19489 for further clinical development, it has some limitations. Though the Indian population showed lower C_{max}, $t_{1/2}$, and AUC and higher T_{max}, the study was not powered enough to conclude the statistical significance. As female participants showed higher exposure (C_{max} and AUC) than male participants, it is important to investigate the potential gender effect on pharmacokinetics with a larger sample size. The analyses of extracted ECGs from the Holter recorder were hampered by an unusual placebo effect and limited sample size.

Recommendations

The current recommendation of the global health agencies is to develop a combination treatment for malaria. ZY-19489 was not effective against the liver stage and sexual stage of the malaria parasite in preclinical studies. It can be combined with other anti-malarial agents. This approach reduces the risk of drug resistance emergence and also ascertains parasite clearance at different lifecycle stages.[3]

Conclusion

ZY-19489 was well-tolerated up to 500 mg once a day for three days. It displayed good oral absorption, dose-proportional increase in exposure, and a long elimination half-life. This study data supports its further development as a novel antimalarial drug candidate.

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Statements

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Conflict of Interest

All authors are employees of Zydus Lifesciences Ltd.

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Appendix

Table S1.Inclusion and Exclusion Criteria

Inclusion Criteria
Demography
Male or female (non-pregnant, non-lactating) aged 18 to 55 years (inclusive) who were contactable and available for the duration of the trial and up to 2 weeks following the end of the study visit.
Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18.5 to 30.0 kg/ m^2 (both inclusive). BMI is an estimate of body weight adjusted for height. It is calculated by dividing the weight in kilograms by the square of the height in meters.
Health Status
Certified as healthy by a comprehensive clinical assessment (detailed medical history, complete physical examination and special investigations)
Screening vital signs: Systolic blood pressure (SBP): 90-140 mmHg Diastolic blood pressure (DBP): 60-90 mmHg Heart rate (HR): 60-90 bpm
QTcF ≤ 450 ms, PR interval ≤ 220 ms
Female subjects with a history of sterility or at least 1-year menopause or use of long-acting non-hormonal contraceptive measures (e.g., intrauterine device) and willing and able to continue contraception for 90 days after administration of study treatment
Male subjects who agreed to use adequate contraception methods during the study and were willing and able to continue contraception for 90 days after administration of study treatment
Regulations
Completion of the written informed consent process prior to undertaking any study-related procedure
Must be willing and able to communicate and participate in the whole study
Exclusion Criteria
A volunteer was not be recruited in the study if he/ she met any of the following conditions:
Medical History and Clinical Status
Haematology, clinical chemistry or urinalysis results at screening that were outside of clinically acceptable laboratory ranges, and were considered clinically significant by the investigator
Participation in any investigational product study within 12 weeks preceding IMP administration
History or presence of diagnosed (by an allergist/ immunologist) or treated (by a physician) food or known drug allergies, or history of anaphylaxis or other severe allergic reactions; subjects with seasonal allergies/ hay fever or allergy to animals or house dust mites that are untreated and asymptomatic could be enrolled in the study based on the investigator's discretion.
History of convulsion (including intravenous drug or vaccine-induced episodes); a medical history of a single febrile convulsion during childhood was not an exclusion criterion.
Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immuno-deficiencies), insulin-dependent and non-insulin-dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma (excluding childhood asthma or mild asthma with preventative asthma medication required less than monthly), epilepsy, or obsessive-compulsive disorder
History of malignancy of any organ system (treated or untreated), within 5 years of screening, regardless of whether there was evidence of local recurrence or metastases
Subjects with a history of schizophrenia, bipolar disease, psychoses, disorders requiring lithium, attempted or planned suicide, or any other severe (disabling) chronic psychiatric diagnosis

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Subjects who had received psychiatric medications within 1 year prior to enrolment, or who had been hospitalised within 5 years prior to enrolment for either a psychiatric illness or due to danger to self or others
History of more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening
History of recurrent headache (e.g., tension-type, cluster or migraine) with a frequency of ≥ 2 episodes per month on average and/ or severe enough to require medical therapy during the 5 years preceding screening
Presence of clinically significant infectious disease or fever (e.g., sublingual temperature ≥ 38.5 °C) within the 14 days prior to enrollment
Evidence of acute illness within the 4 weeks prior to screening that the investigator deemed may compromise subject safety
A significant intercurrent disease of any type, in particular, liver, renal, cardiac, pulmonary, neurologic, gastrointestinal, rheumatologic, or autoimmune disease by history, physical examination, and/ or laboratory studies including urinalysis
A clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion (e.g., gastrectomy, diarrhoea)
Blood donation of any volume within 1 month before IMP administration, or participation in any research study involving blood sampling (more than 450 mL/unit of blood), or blood donation to a blood bank during the 12 weeks prior to IMP administration
Medical requirement for intravenous immunoglobulin or blood transfusions within 3 months prior to enrollment
History or presence of alcohol abuse (alcohol consumption of more than 40 g/ 4 units/ 4 standard drinks per day), or drug habituation, or any prior intravenous usage of an illicit substance in the past one year
Tobacco use of more than 5 cigarettes or equivalent per day for the last one year, and inability to stop smoking for the duration of the clinical unit confinement
Female subject who was breastfeeding or pregnant or was found positive on a pregnancy test.
Interfering Substances
Any vaccination within the last 28 days and planned vaccination till the final study visit
Any corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past 3 months; any subject currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past year
Ingestion of any poppy seeds within the 24 hours prior to screening
Consumption of beverages or food containing xanthine bases including Red Bull, chocolate, coffee etc. within the 48 hours prior to enrollment
Unwillingness to abstain from the consumption of grapefruit or Seville oranges from 7 days prior to enrollment until the end of the study
Use of prescription drugs (excluding contraceptives) or non-prescription drugs or herbal supplements (such as St John's Wort), within 14 days or 5 half-lives (whichever is longer) prior to IMP dosing. Limited use of other non-prescription medications or dietary supplements, not believed to affect subject safety or the overall results of the study, may be permitted on a case-by-case basis following approval by the sponsor in consultation with the investigator. The subjects were requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the study.
General Conditions
Any subject who, in the judgement of the investigator, was likely to be non-compliant during the study, or was unable to cooperate because of a language problem or poor mental development
Any subject who was the principal investigator, sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study

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Any subject without good peripheral venous access
Biological Status
Positive results on any of the following tests: hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV) antibodies, anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti-HIV2 Ab)
Positive urine drug test
Positive urine alcohol/ breath alcohol test
Specific to the Study
History or presence of any of the following cardiac conditions: known structural cardiac abnormalities, family history of long QT syndrome, cardiac syncope or recurrent idiopathic syncope, exercise-related clinically significant cardiac events
Any clinically important abnormalities in rhythm, conduction or morphology of resting ECG that may interfere with the interpretation of QTc interval changes. This includes subjects with any of the following (at screening):
Sinus node dysfunction
Complete bundle branch block
Abnormal T wave morphology
Any other ECG abnormalities in the standard 12-lead ECG, that in the opinion of the investigator, might interfere with the ECG analysis

Table S2.Details of Adverse Events with System Organ Class and Preferred Term: Single Dose Cohort

System Organ Class Term/ Preferred Term	ZY-19489 (450 mg) (N = 6) n (%) [E]	Placebo (N = 2) n (%) [E]	Total (N = 8) n (%) [E]
Number of subjects with at least one adverse event	5 (83.33) [8]	0 (0.00) [0]	5 (62.50) [8]
Infections and infestations	3 (50.00) [4]	0 (0.00) [0]	3 (37.50) [4]
Dengue fever	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Nasopharyngitis	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Pyuria	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Typhoid fever	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Investigations	2 (33.33) [3]	0 (0.00) [0]	2 (25.00) [3]
Alanine aminotransferase increased	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Gamma-glutamyl transferase increased	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Transaminases increased	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Nervous system disorders	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]
Headache	1 (16.67) [1]	0 (0.00) [0]	1 (12.50) [1]

N: Number of subjects in the safety population in each arm group which is used as the denominator to calculate percentages; n: Number of subjects in each arm group in a specific category; E: Events

If a subject has multiple occurrences of an AE, the subject is presented only once for the corresponding AE.

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Table S3.Details of Adverse Events with System Organ Class and Preferred Term: Multiple Dose Cohort

System Organ Class Term/ Preferred Term	ZY-19489 (300 mg) (N = 6) n (%) [E]	ZY-19489 (500 mg) (N = 6) n (%) [E]	Placebo (N = 4) n (%) [E]	Total (N = 16) n (%) [E]
Number of subjects with at least one adverse event	2 (33.33) [5]	5 (83.33) [6]	2 (50.00) [6]	9 (56.25) [17]
Gastrointestinal disorders	1 (16.67) [2]	2 (33.33) [2]	1 (25.00) [1]	4 (25.00) [5]
Abdominal pain upper	0 (0.00) [0]	1 (16.67) [1]	0 (0.00) [0]	1 (6.25) [1]
Nausea	1 (16.67) [1]	0 (0.00) [0]	0 (0.00) [0]	1 (6.25) [1]
Vomiting	1 (16.67) [1]	1 (16.67) [1]	1 (25.00) [1]	3 (18.75) [3]
Infections and infestations	0 (0.00) [0]	0 (0.00) [0]	1 (25.00) [1]	1 (6.25) [1]
Nasopharyngitis	0 (0.00) [0]	0 (0.00) [0]	1 (25.00) [1]	1 (6.25) [1]
Investigations	2 (33.33) [2]	3 (50.00) [3]	2 (50.00) [2]	7 (43.75) [7]
Alanine aminotransferase increased	0 (0.00) [0]	2 (33.33) [2]	1 (25.00) [1]	3 (18.75) [3]
Haemoglobin decreased	1 (16.67) [1]	0 (0.00) [0]	1 (25.00) [1]	2 (12.50) [2]
Hepatic enzyme increased	1 (16.67) [1]	0 (0.00) [0]	0 (0.00) [0]	1 (6.25) [1]
Transaminases increased	0 (0.00) [0]	1 (16.67) [1]	0 (0.00) [0]	1 (6.25) [1]
Musculoskeletal and connective tissue disorders	1 (16.67) [1]	0 (0.00) [0]	0 (0.00) [0]	1 (6.25) [1]
Pain in extremity	1 (16.67) [1]	0 (0.00) [0]	0 (0.00) [0]	1 (6.25) [1]
Nervous system disorders	0 (0.00) [0]	1 (16.67) [1]	2 (50.00) [2]	3 (18.75) [3]
Headache	0 (0.00) [0]	1 (16.67) [1]	2 (50.00) [2]	3 (18.75) [3]

N: Number of subjects in the safety population in each arm group which is used as the denominator to calculate percentages; n: Number of subjects in each arm group in a specific category; E: Events.

If a subject has multiple occurrences of an AE, the subject is presented only once for the corresponding AE.

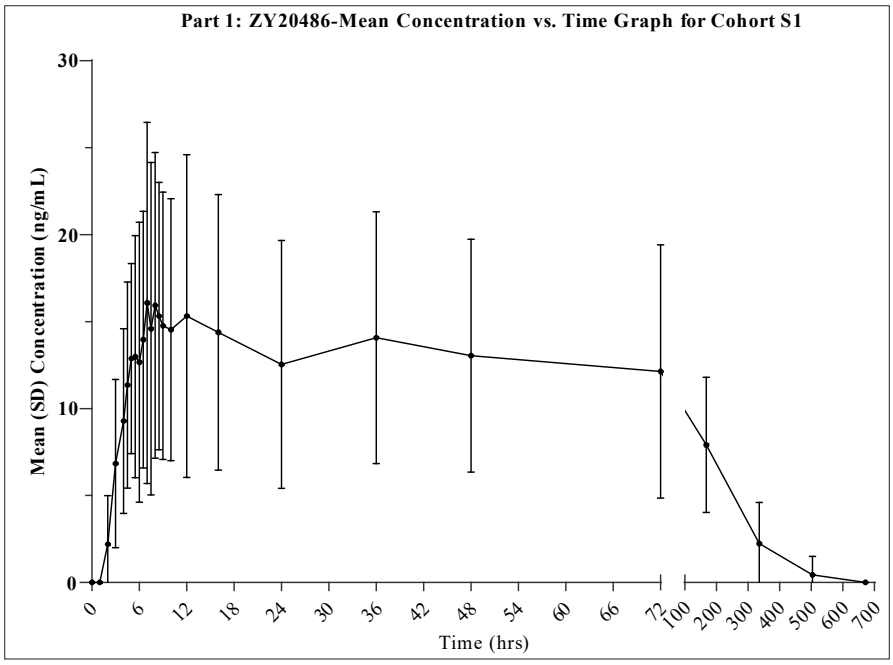

Figure S1.Mean (± Standard Error of the Mean) Concentration-time Profiles Metabolites after Administration of 450 mg Single Dose to Healthy Human Participants

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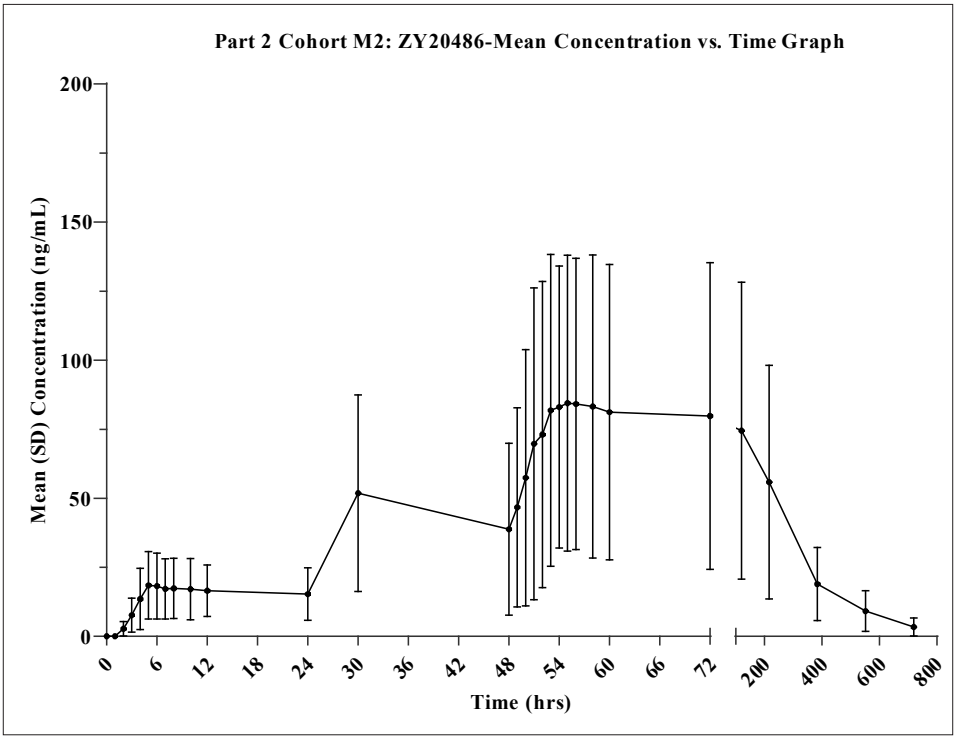

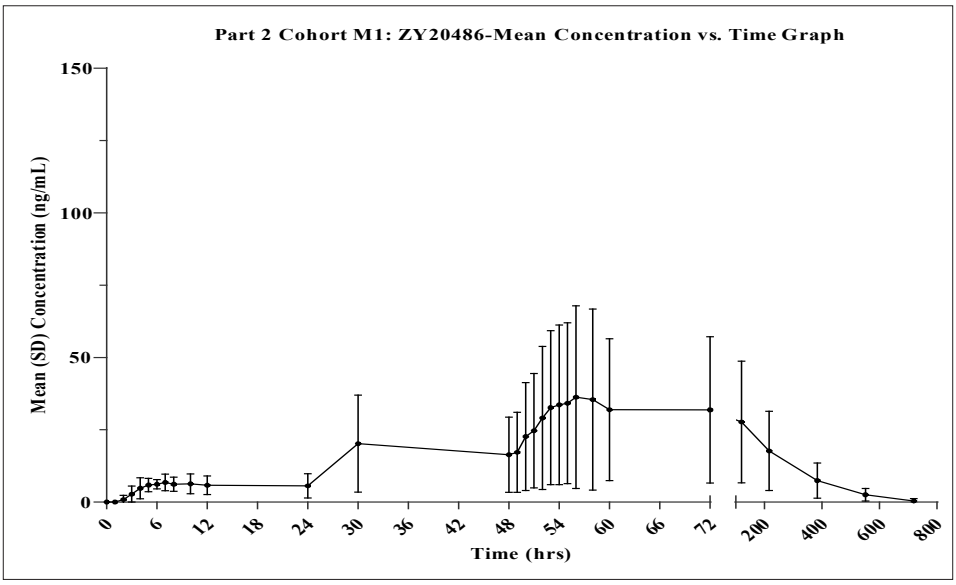

Figure S2.Mean (± Standard Deviation of the Mean) Concentration-time Profiles of Metabolites of 300 and 500 mg Once A Day for Three Days given to Healthy Human Participants

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