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PHARMACOKINETICS AND DISPOSITION

Interaction potential of the dual orexin receptor antagonist ACT-541468 with CYP3A4 and food: results from two interaction studies

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Abstract

Purpose ACT-541468 is a novel dual orexin receptor antagonist (DORA) under development for the treatment of insomnia. In vitro studies suggested a significant role of CYP3A4 in ACT-541468 metabolism and an impact on CYP3A4 activity.

Methods Subsequently, two clinical cross-over studies investigated the victim ($n = 14$ healthy subjects) and perpetrator ($n = 20$) potential of 25 mg ACT-541468 with respect to CYP3A4. The effect of food intake on the pharmacokinetics of ACT-541468 was also investigated.

Results Moderate CYP3A4 inhibition by diltiazem (240 mg/day) increased the C_{max} and $AUC_{0-\infty}$ of ACT-541468 by 1.4-fold (90% confidence interval (CI): 1.2–1.6) and 2.4-fold (90% CI: 2.0–2.8), respectively, and prolonged $t_{½}$ by 80% (90% CI: 60–90) without affecting t_{max}. Single- and multiple-dose administration of 25 mg ACT-541468 had no impact on the pharmacokinetics of the sensitive substrate midazolam and its main metabolite 1-hydroxy midazolam indicated by 90% CI of the geometric mean ratios of C_{max} and AUC within bioequivalence criteria and by an unchanged t_{max}. After a high-fat high-calorie breakfast, the pharmacokinetic profile of 25 mg ACT-541468 showed a decrease of C_{max} by 24% (90% CI: 17–31) and a delay of t_{max} by approximately 2 h (90% CI: 1.4–2.4), whereas $t_{½}$ and AUC_{0-24} remained essentially unchanged. ACT-541468 given alone or in combination with diltiazem, midazolam, or food was safe and well tolerated.

Conclusions Overall, ACT-541468 has been determined as CYP3A4 substrate but without any perpetrator drug–drug interaction potential regarding CYP3A4 in humans. Food affected ACT-541468 absorption without modifying overall exposure.

Keywords ACT-541468 · CYP3A4 · Food · Diltiazem · Midazolam · Pharmacokinetics

Introduction

The orexin system, discovered in 1998, involves two neuropeptides orexin A and orexin B that are synthesized in the lateral hypothalamic area which projects to various regions in the brain and that binds two G protein-coupled receptors orexin-1 (OX1) and orexin-2 (OX2) [1, 2]. This system plays a central role in the regulation of arousal and sleep–wake balance [3–5]. To date, a number of dual orexin receptor antagonists (DORAs) have entered clinical development for the treatment of sleep disorders: almorexant [6, 7], ACT-462206 [8], SB-649868 [9], lemborexant [10], filorexant [11], and suvorexant [12]. In 2014, suvorexant was granted market authorization in the US and Japan (Belsomra®) for the treatment of insomnia.

ACT-541468 ((S)-(2-(5-chloro-4-methyl-1H-benzo[*d*]-imidazol-2-yl)-2-methylpyrrolidin-1-yl)(5 methoxy-2-(2H-1,2,3-triazol-2-yl)phenyl)methanone) is a novel DORA [13] that is currently developed for the treatment of insomnia. The chemical structure is provided in the supplemental Fig. S1. In the first-in-human study, ACT-541468 was characterized by quick absorption and elimination, with median time to reach maximum concentration (t_{max}) of 0.8–2.8 h and geometric mean elimination half-life ($t_{½}$) of 5.9 to 8.8 h. Dose-related sedative effects were observed indicating pharmacokinetic

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(PK) and pharmacodynamic (PD) profiles suitable for a sleep-promoting compound [14].

The role of cytochrome P450 (CYP) 3A4 in the metabolism of DORAs has been extensively evaluated in preclinical and clinical development stages and it was shown that this CYP is the main enzyme involved in the metabolism of suvorexant [15], almorexant [16], and SB-649868 [17]. In vitro studies were performed to quantify the role of CYP3A4 in ACT-541468 metabolism and to assess its inhibitory and inductive effects on CYP3A4 activity (data on file). Based on these data, > 80% of ACT-541468 clearance was predicted to be mediated by CYP3A4. In human liver microsomes, CYP3A4 activity (i.e., midazolam and testosterone hydroxylation) was inhibited with an IC_{50} of approximately 10 μM. Therefore, two clinical drug–drug interaction (DDI) studies have been conducted to assess the victim and perpetrator DDI potential of ACT-541468 with respect to CYP3A4. In addition, the effect of a high-fat high-calorie breakfast on the PK of ACT-541468 was assessed to better define clinical instructions of use in the pivotal studies.

Methods

Each study was approved by the German health authority (BfArM) and by the Ethics committee (Ethikkommission II, Bad Segeberg, Germany). They were both conducted in accordance with the Declaration of Helsinki principles, International Council for Harmonization Good Clinical Practice guidelines, and applicable regulations and laws. All subjects provided written informed consent prior to any screening procedures.

Study designs

Study 1 (Fig. 1a) was a single-center, open-label, randomized, cross-over study in 14 subjects (7 subjects per sequence) to investigate the effect of the moderate CYP3A4 inhibitor diltiazem on the PK of ACT-541468. Each subject received a single oral dose of 25 mg ACT-541468 under fasting conditions alone (Treatment A) or on Day 4 of a 7-day multiple, oral dose regimen of 240 mg diltiazem once daily for 7 days (Treatment B), i.e., at steady-state exposure of diltiazem [18, 19].

Study 2 (Fig. 1b) was a single-center, open-label, fixed-sequence study in 20 subjects to investigate the effect of ACT-541468 on the PK of the CYP3A4 index substrate midazolam [20] and its main metabolite 1-hydroxy midazolam (1-OH midazolam) as well as the effect of a high-fat high-calorie breakfast on the PK of ACT-541468. Each subject received a single, oral dose of 2 mg midazolam under fasting conditions alone (Treatment A) and in combination with single (Treatment B) and multiple (Treatment D) oral doses of 25 mg ACT-541468 once daily for 5 days. For the assessment of food effect on the PK of ACT-541468, subjects were given a high-fat high-calorie breakfast 30 min prior to the administration of a single dose of 25 mg ACT-541468 as per Food and Drug Administration (FDA) guidance [21] and European Medicines Agency (EMA) guidelines [22] during Treatment C. ACT-541468 PK parameters under fasting conditions were assessed during Treatment B.

The safety and tolerability of ACT-541468 administered alone or in combination with either diltiazem or midazolam was also assessed.

For these two bio-comparison studies, the within-subject standard deviation (SD_w) on log scale observed for area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$, study 1) or AUC from 0 to 24 h after administration (AUC_{0-24}, study 2) and maximum observed plasma concentration (C_{max}) were estimated from previous studies for ACT-541468 [14] and for midazolam/1-OH midazolam [23]. For ACT-541468, SD_w was 0.30 for AUC and 0.15 for C_{max}. For midazolam and 1-OH midazolam, SD_w was 0.44 and 0.41, respectively, for AUC and 0.36 and 0.44, respectively, for C_{max}. In study 1, it was estimated that, with a sample size of 12 subjects, the 90% confidence interval (CI) of the geometric mean ratio (GMR) "ACT 541468 + diltiazem/ACT-541468" would be 0.86–1.17 for $AUC_{0-\infty}$ and 0.92–1.08 for C_{max}. In study 2, corresponding data for "midazolam + ACT-541468/midazolam" were, with a sample size of 16 subjects, 0.66–1.52 for AUC_{0-24} and 0.71–1.41 for C_{max} of midazolam and 0.68–1.52 for AUC_{0-24} and 0.66–1.52 for C_{max} of 1-OH midazolam.

To compensate for any potential dropouts, 14 subjects (study 1) and 20 subjects (study 2) were enrolled to achieve a minimum of 12 and 16 subjects, respectively, completing the studies per protocol.

Subjects

Non-smoking, healthy male adults 18–45 years old with a body mass index (BMI) of 18–28 kg/m^2 (study 1) or 18–30 kg/m^2 (study 2) were eligible for study entry. To be eligible, the results of the self-administered modified Swiss Narcolepsy Scale questionnaire [24] had to show no signs of narcolepsy/cataplexy.

Consumption of nicotine, alcohol, or food and beverages that could influence CYP3A activity (e.g., grapefruit or xanthine-containing beverages) was forbidden. Concomitant therapy was prohibited unless required for the treatment of an adverse event (AE).

Blood sampling and bioanalysis of ACT-541468, midazolam, and 1-OH midazolam concentrations in plasma

Serial blood samples were collected in EDTA tubes for 96 h (study 1) and for 24 h (study 2) for the determination of ACT-

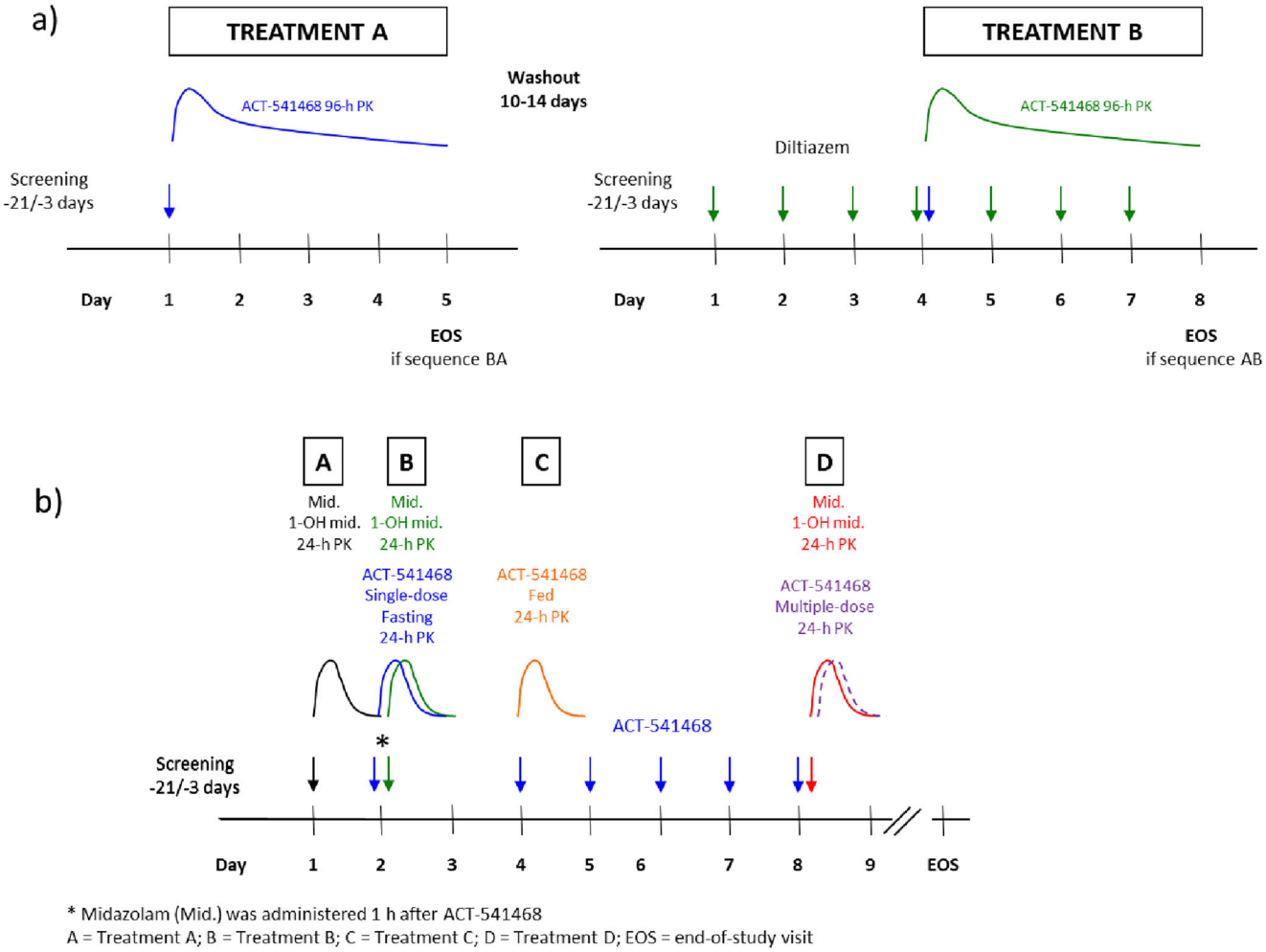

Fig. 1 Schematic presentation of the clinical study design for (**a**) study 1 (CYP3A4 victim potential of ACT-541468) and (**b**) study 2 (CYP3A4 perpetrator potential of ACT-541468)

541468 (study 1 and 2) and midazolam and 1-OH midazolam (study 2) plasma concentrations. In study 2, pre-dose samples from day 5 to day 7 were also collected to assess the trough plasma concentrations (C_{trough}) of ACT-541468. After centrifugation, plasma was transferred to polypropylene tubes and stored at − 20 °C or below.

Concentrations of ACT-541468, midazolam, and 1-OH midazolam in plasma were measured using previously described validated liquid chromatography methods with tandem mass spectrometry (LC-MS/MS) [14, 25]. The lower limit of quantification (LLOQ) was 0.5 ng/mL for ACT-541468 and 0.1 ng/mL for both midazolam and 1-OH midazolam. For ACT-541468, inter-batch precision expressed as coefficient of variation (%CV) was ≤ 5.4% (study 1) and ≤ 4.4% (study 2), whereas the inter-batch accuracy expressed as relative deviation from nominal value (%RD) ranged from − 6.2% to − 1.9% (study 1) and from − 0.8 to 3.2% (study 2). For midazolam and 1-OH midazolam, %CV was ≤ 6.0% and ≤ 6.6%, respectively, whereas %RD ranged from − 1.9 to 2.3% and from − 3.7 to 0.3%, respectively.

Pharmacokinetic calculations

The PK parameters of ACT-541468 in both studies as well as of midazolam and 1-OH midazolam (study 2) were obtained by non-compartmental analysis using Phoenix WinNonlin (version 6.4; Pharsight Corporation, Mountain View, CA, USA). The measured individual plasma concentrations of each analyte were used to directly obtain C_{max} and t_{max}. AUC values were calculated according to the linear trapezoidal rule, using the measured concentration values above the LLOQ, without any weighting. The $t_{½}$ of all analytes was calculated as follows: $t_{½} = \ln(2)/\lambda_z$, where λ_z represents the terminal elimination rate constant. Assessment of steady-state concentrations of ACT-541468 (study 2) was based on C_{trough} data.

Statistical analysis of pharmacokinetic variables

The PK analysis was based on the per-protocol analysis set, which included all subjects that completed the dosing

as per protocol. PK variables were analyzed providing geometric means and corresponding 95% CI for AUC, C_{max}, and $t_{½}$ whereas the median and range were used for t_{max}.

The effects of diltiazem and food on AUC, C_{max}, and $t_{½}$ of ACT-541468 were explored using the GMR and 90% CIs of "ACT-541468 + diltiazem" (study 1, Treatment B) or "ACT-541468 + food" (study 2, Treatment C) with ACT-541468 alone as reference treatment.

Similarly, the effect of ACT-541468 on AUC, C_{max}, and $t_{½}$ of midazolam and 1-OH midazolam was explored using the GMR and 90% CIs of "midazolam + single-dose ACT-541468" (study 2, Treatment B) or "midazolam + multiple-dose ACT-541468" (study 2, Treatment D) with midazolam alone as reference treatment.

The log-transformed values were analyzed by mixed-effect models including treatment, sequence (study 1 only), and period as fixed effects and subject as random effect. Differences between treatments for t_{max} were explored using the Wilcoxon signed-rank test providing the median differences and corresponding 90% CI.

All statistical analyses were performed using SAS® version 9.3 (SAS Institute, Cary, NC, USA).

Safety and tolerability

Safety and tolerability were assessed based on vital sign, 12-lead electrocardiogram (ECG), physical examination, clinical chemistry, hematology, and AE data. The all-treated analysis set, which included all subjects who received at least one dose of treatment, was used for the safety analysis.

Results

Study population

A total of 14 (study 1) and 20 (study 2) subjects were enrolled in this study and were included in the all-treated analysis set. All subjects completed the studies as per protocol except for one subject in study 1 who discontinued the treatment because he did not come to the study site to receive diltiazem on Day 2 and was thus excluded from the per-protocol analysis set. In both studies, all subjects were Caucasian except one per study who was Asian. Demographic characteristics (age and BMI) were similar across the Treatment sequences in study 1 and between both studies (Table 1). None of the subjects deviated from any inclusion/exclusion criteria.

Pharmacokinetic results

Effect of the moderate CYP3A4 inhibitor diltiazem on the PK of ACT-541468

Concomitant administration of 240 mg diltiazem once daily increased C_{max} of ACT-541468 by 1.4-fold and $AUC_{0-\infty}$ by 2.4-fold (Figure 2, Table 2), indicating ACT-541468 as sensitive substrate of CYP3A4 in humans according to FDA guidance [26]. ACT-541468 $t_{½}$ was prolonged by 5.4 h whereas t_{max} was shortened by 15 min (Fig. 2, Table 2).

Effect of ACT-541468 on the PK of the CYP3A4 index substrate midazolam and its metabolite 1-OH midazolam

Steady-state plasma concentrations of ACT-541468 were reached on the second day of multiple-dose administration based on C_{trough} data (Fig. S2a). The PK profile and parameters of ACT-541468 were similar after single- and multiple-dose administration (Fig. S2b, Table 3) indicating no relevant accumulation.

After single-dose ACT-541468 administration, geometric mean C_{max} and AUC_{0-24} of midazolam were increased by 20 and 11%, respectively (Fig. 3a and S3a, Table 3), whereas they were increased by only 5% for 1-OH midazolam (Fig. 3b and S3b, Table 3). As for C_{max} and AUC_{0-24}, $t_{½}$ and t_{max} of both analytes were essentially unchanged when compared to midazolam given alone (Fig. 3a, b, S3a, and S3b, Table 3).

After multiple-dose ACT-541468 administration, geometric mean C_{max} and AUC_{0-24} of midazolam were decreased by 6.4 and 2.5%, respectively (Fig. 3a and S3a, Table 3), whereas 1-OH midazolam C_{max} was only decreased by 1.8% and AUC_{0-24} remained unchanged (Fig. 4b and S3b, Table 4). Accordingly, after single-dose ACT-541468 administration, $t_{½}$ and t_{max} of both analytes were essentially unchanged when compared to midazolam given alone (Fig. 3a, b, S3a, and S3b, Table 3).

Overall, the PK of midazolam and 1-OH midazolam were essentially unaffected by single- and multiple-dose administration of 25 mg ACT-541468.

Effect of food on the PK of ACT-541468

In the presence of food, C_{max} of ACT-541468 was decreased by 24% and t_{max} was delayed by approximately 2 h, whereas $t_{½}$ and AUC_{0-24} remained essentially unchanged (increase of 2.6 and 3.2%, respectively) (Fig. 4, Table 4).

Safety and tolerability

Single oral doses of 25 mg ACT-541468 alone or in combination with multiple oral doses of 240 mg diltiazem, high-fat high-calorie breakfast, and single oral dose of 2 mg

Table 1 Baseline demographic characteristics of study 1 and study 2

Study 1			
	Treatment sequence AB ($n = 7$)	Treatment sequence BA ($n = 7$)	Overall ($n = 14$)
Age (years) mean (SD)	28.9 (6.8)	30.9 (12.1)	29.9 (9.5)
Race, *n* (%)			
Asian	1 (14.3%)		1 (7.1%)
White	6 (85.7%)	7 (100%)	13 (92.9%)
Weight (kg) mean (SD)	80.3 (13.6)	75.8 (7.3)	78.1 (10.7)
Height (cm) mean (SD)	181.9 (8.6)	180.6 (5.2)	181.2 (6.9)
BMI (kg/m^2) mean (SD)	24.2 (3.1)	23.4 (3.1)	23.8 (3.0)

Study 2	
	Overall ($n = 20$)
Age (years) mean (SD)	28.9 (8.5)
Race, *n* (%)	
Asian	1 (5.0%)
White	19 (95.0%)
Weight (kg) mean (SD)	78.5 (11.3)
Height (cm) mean (SD)	178.3 (7.3)
BMI (kg/m^2) mean (SD)	24.6 (2.5)

Arithmetic mean is presented. Treatment sequences: AB = ACT-541468 alone → diltiazem plus ACT-541468, BA = diltiazem plus ACT-541468 → ACT-541468 alone

BMI body mass index, *SD* standard deviation

midazolam were safe and tolerated. There were no clinically relevant effects on vital sign, ECG, or laboratory variables. None of the AEs were serious or led to discontinuation from the study. All treatment-emergent AEs were transient and resolved without sequelae before the end of the studies.

In both studies, the most frequently reported AEs were somnolence and headache. Somnolence was reported by each subject exposed to ACT-541468 alone or in combination with either diltiazem, food, or midazolam. These AEs of somnolence were rated as mild (33.3%), moderate (63.2%), or severe

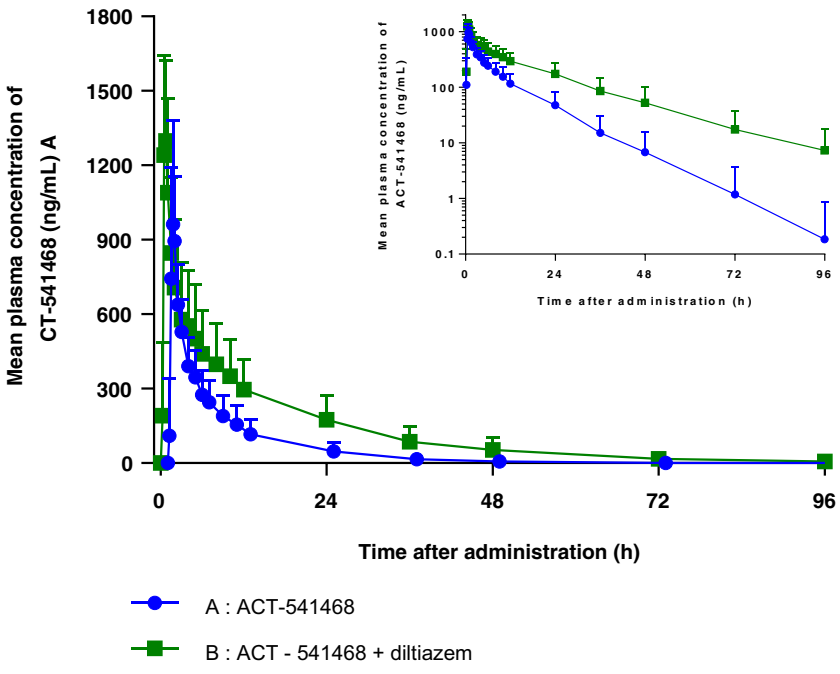

Fig. 2 Arithmetic mean (± SD) plasma concentration–time profile of ACT-541468 after administration of ACT-541468 alone and concomitantly with diltiazem ($n = 13$) on a linear and semi-logarithmic scale

Table 2 Summary of pharmacokinetic parameters of ACT-541468 when administered alone or concomitantly with diltiazem (study 1, $n = 13$)

	Treatment A	Treatment B	GMR or mean differences[a]
C_{max} (ng/mL)	991 (790, 1245)	1406 (1221, 1618)	1.41 (1.23, 1.61)
t_{max} (h)	0.75 (0.5, 1.5)	0.50 (0.5, 0.8)	− 0.24 (− 0.38, − 0.11)
$t_{½}$ (h)	6.9 (5.5, 8.7)	12.3 (9.7, 15.6)	1.76 (1.63, 1.91)
$AUC_{0-\infty}$ (ng h/mL)	4856 (3669, 6426)	11590 (8882, 15124)	2.35 (1.96, 2.83)

Treatments: A = ACT-541468 alone, B = diltiazem + ACT-541468. Data for Treatment A and Treatment B are geometric mean (95% CI) except for t_{max}, for which data are median (range)

$AUC_{0-\infty}$ area under the plasma concentration–time curve from time zero to infinity, *CI* confidence interval, *C_{max}* maximum plasma concentration, *GMR* geometric mean ratio, *$t_{½}$* terminal elimination half-life, *t_{max}* time to reach maximum plasma concentration

[a] Data for Treatment B/Treatment A are ratio of the geometric means (90% CI) except for t_{max}, for which data are median differences (90% CI)

(3.5%) based on pooled analysis from both studies. The second most common AE was headache reported by 50% of the subjects in study 1 mostly after the administration of diltiazem alone and by 25% of the subjects in study 2, exclusively after the administration of midazolam alone. These AEs were mild (71.4%) or moderate (28.6%) in intensity.

Discussion

In line with the in vitro data suggesting ACT-541468 as CYP3A4 substrate, the first clinical study shows that the single-dose PK of ACT-541468 are modulated by the moderate CYP3A4 inhibitor diltiazem. However, the second clinical study shows that ACT-541468 itself does not modulate the PK of the CYP3A4 index substrate midazolam despite some inhibition/induction potential identified in vitro. This study also shows that a high-fat high-calorie breakfast has no impact on the overall exposure to ACT-541468 but modulates C_{max} and t_{max} to some extent.

Both exploratory bio-comparison studies were conducted in line with the applicable guidelines in terms of design and choice of interacting compounds/meal content for interaction with food [20–22]. ACT-541468 was administered at a dose of 25 mg (single and multiple) since it is considered as potential therapeutically relevant dose for the treatment of insomnia triggering the desired PD effects with the appropriate onset

Table 3 Summary of pharmacokinetic parameters of ACT-541468, midazolam, and 1-OH midazolam when midazolam is administered alone (Treatment A) or concomitantly with single-dose (Treatment B), and multiple dose (Treatment D) ACT-541468 (study 2, $n = 20$)

	Treatment A	Treatment B	Treatment D	GMR or median differences[a] Treatment B/Treatment A	GMR or median differences[a] Treatment D/Treatment A
ACT-541468					
C_{max} (ng/mL)		716 (613, 836)	783 (655, 935)		
t_{max} (h)		1.0 (0.5, 3.0)	1.3 (0.5, 3.0)		
$t_{½}$ (h)		7.6 (6.3, 9.2)	8.5 (7.2, 10.1)		
AUC_{0-24} (ng h/mL)		4252 (3114, 5295)	4917 (3924, 6162)		
Midazolam					
C_{max} (ng/mL)	12.1 (10.3, 14.3)	14.6 (12.3, 17.1)	11.3 (9.5, 13.6)	1.20 (1.07, 1.34)	0.94 (0.83, 1.05)
t_{max} (h)	0.5 (0.3, 1.0)	0.5 (0.3, 0.8)	0.5 (0.5, 1.0)	0.00 (− 0.13, 0.00)	0.00 (0.00, 0.13)
$t_{½}$ (h)	5.2 (4.1, 6.6)	5.5 (4.6, 6.7)	4.6 (3.7, 5.6)	1.05 (0.93, 1.20)	0.88 (0.78, 0.98)
AUC_{0-24} (ng h/mL)	28.1 (23.6, 33.5)	31.1 (26.1, 37.0)	27.4 (22.7, 33.1)	1.11 (1.03, 1.19)	0.98 (0.91, 1.05)
1-OH midazolam					
C_{max} (ng/mL)	4.4 (3.6, 5.3)	4.6 (3.7, 5.6)	4.3 (3.4, 5.4)	1.05 (0.93, 1.19)	0.98 (0.84, 1.15)
t_{max} (h)	0.5 (0.5, 1.0)	0.5 (0.3, 0.8)	0.5 (0.5, 1.0)	0.00 (− 0.13, 0.00)	0.00 (0.00, 0.13)
$t_{½}$ (h)	3.7 (2.6, 5.3)	3.7 (2.8, 4.9)	3.3 (2.4, 4.5)	1.00 (0.79, 1.27)	0.89 (0.70, 1.14)
AUC_{0-24} (ng h/mL)	8.1 (6.6, 9.9)	8.5 (6.9, 10.4)	8.1 (6.9, 10.4)	1.05 (0.98, 1.12)	1.01 (0.98, 1.12)

Treatments: A = 2 mg midazolam; B = 2 mg midazolam 1 h after a single dose of 25 mg ACT-541468; D = 2 mg midazolam concomitantly with 25 mg ACT-541468 after multiple-dose (5 days) 25 mg ACT-541468. Data for Treatment A, Treatment B, and Treatment D are geometric mean (95% CI) except for t_{max}, for which data are median (range)

AUC_{0-24} area under the plasma concentration–time curve from time zero to 24 h, *CI* confidence interval, *C_{max}* maximum plasma concentration, *$t_{½}$* terminal elimination half-life, *t_{max}* time to reach maximum plasma concentration

[a] Data for Treatment B/Treatment A and Treatment D/Treatment A are ratio of the geometric means (90% CI) except for t_{max}, for which data are median differences (90% CI)

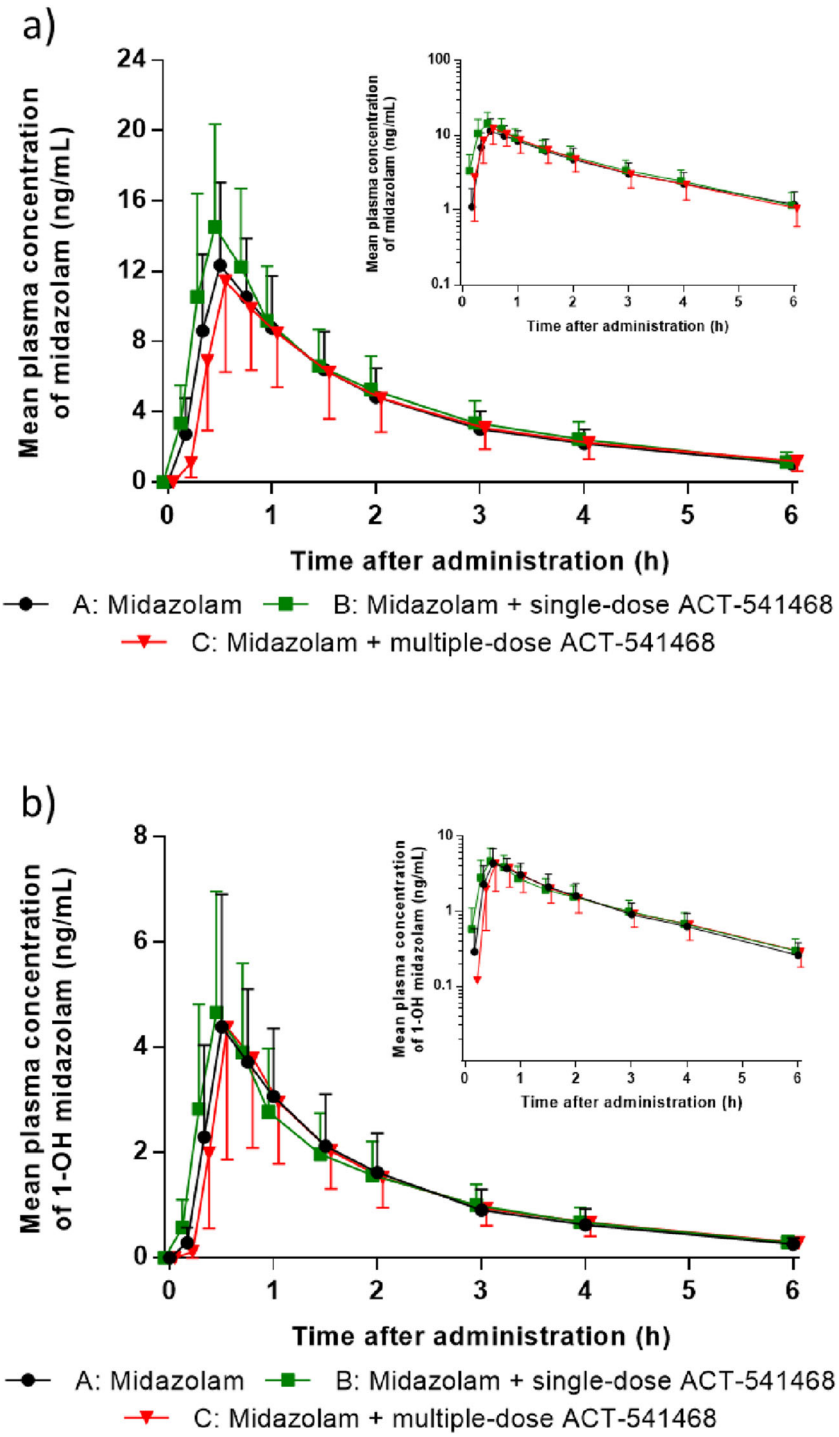

Fig. 3 Arithmetic mean (± SD) plasma concentration–time profile of (**a**) midazolam and (**b**) 1-OH midazolam after administration of midazolam alone, 1 h after single-dose ACT-541468, and after multiple-dose 25 mg ACT-541468 from time 0 to 6 h post-dose on a linear and semi-logarithmic scale ($n = 20$)

and duration of action [14]. Both studies included only male subjects to have a more homogenous population.

ACT-541468 victim potential in the context of moderate CYP3A4 inhibition

To evaluate the relevance of the effect of CYP3A4 on ACT-541468 metabolism in humans, the moderate CYP3A4 inhibitor diltiazem was administered once daily at the therapeutic dose of 240 mg for 7 days to reach steady-state conditions [18] and maintain continuous inhibition during the elimination phase of ACT-541468 [27, 28]. In previous DDI studies, steady-state conditions were attained on the third day of diltiazem administration [28] and maximal CYP3A4 inhibition was obtained after 2 days of administration [18]. The same dose of diltiazem was used for the evaluation of suvorexant DDI potential with a moderate CYP3A4 inhibitor [29]. After concomitant administration with diltiazem, C_{max}, $AUC_{0-\infty}$,

Table 4 Summary of pharmacokinetic parameters of ACT-541468 when administered under fasting or fed conditions (study 2, $n = 20$)

	Treatment B	Treatment C	GMR or mean differences[b]
C_{max} (ng/mL)	716 (613, 836)	542 (470, 625)	0.76 (0.69, 0.83)
t_{max} (h)	0.97 (0.5, 3.0)	3.00 (1.0, 12.0)	1.88 (1.38, 2.39)
$t_{½}$ (h)	7.6 (6.3, 9.2)	7.5[a] (6.3, 8.9)	0.97[a] (0.89, 1.07)
$AUC_{0–24}$ (ng h/mL)	4252 (3414, 5295)	4388 (3580, 5378)	1.03 (0.99, 1.08)

Treatments: B = 25 mg ACT-541468 under fasting conditions, C = 25 mg ACT-541468 under fed conditions. Data for treatment B and treatment C are geometric mean (95% CI) except for t_{max}, for which data are median (range)

$AUC_{0–24}$ area under the plasma concentration–time curve from time zero to 24 h, *CI* confidence interval, *C_{max}* maximum plasma concentration, *GMR* geometric mean ratio, *$t_{½}$* terminal elimination half-life, *t_{max}* time to reach maximum plasma concentration

[a] One subject was excluded for summary statistics of $t_{½}$. Due to an unreliable t_{max} value (12 h post-dose) under fed conditions, $t_{½}$ could not be determined according to internal standard operating procedures. Thus, $n = 19$ for summary statistics for $t_{½}$ and for exploration of the effect of food

[b] Data for Treatment C/Treatment B are ratio of the geometric means (90% CI) except for t_{max}, for which data are median differences (90% CI)

and $t_{½}$ of ACT-541468 were increased by 1.4-, 2.4-, and 1.8-fold, respectively. The increase of C_{max} and AUC as well as the longer $t_{½}$ indicates that both absorption and elimination are affected by moderate CYP3A4 inhibition. The 2.4-fold $AUC_{0–\infty}$ increase of ACT-541468 upon co-administration of the moderate CYP3A4 inhibitor diltiazem is contained in the 2- to 5-fold range and hence, ACT-541468 may be considered as "sensitive" CYP3A4 substrate according to FDA guidance in 2012 [26]/EMA guidelines [22] on clinical DDI studies. The larger $AUC_{0–\infty}$ of ACT-541468 in the presence of diltiazem did not translate into an increased intensity classification of somnolence when compared to ACT-541468 given alone. However, the longer $t_{½}$ of ACT-541468 could translate in a longer duration of somnolence.

Clinical DDI studies with other DORAs are restricted to suvorexant [29] and almorexant [16]. Here, the AUC of suvorexant (20 mg) and almorexant (100 mg) was increased by 2.1- and 3.7-fold, respectively, upon multiple-dose administration of 240 mg or 300 mg diltiazem, respectively. Similarly, C_{max} was increased by 1.2- and 3.2-fold for suvorexant and almorexant, respectively.

At the time of study conduct, both FDA guidance and EMA guidelines recommended the use of a strong CYP3A4 inhibitor (so-called perpetrator index in the new FDA draft guidance [20]) to assess the DDI potential with CYP3A4 [22, 26]. However, this study was conducted with a moderate CYP3A4 inhibitor since concomitant administration of ACT-541468 at a therapeutically relevant dose with a strong

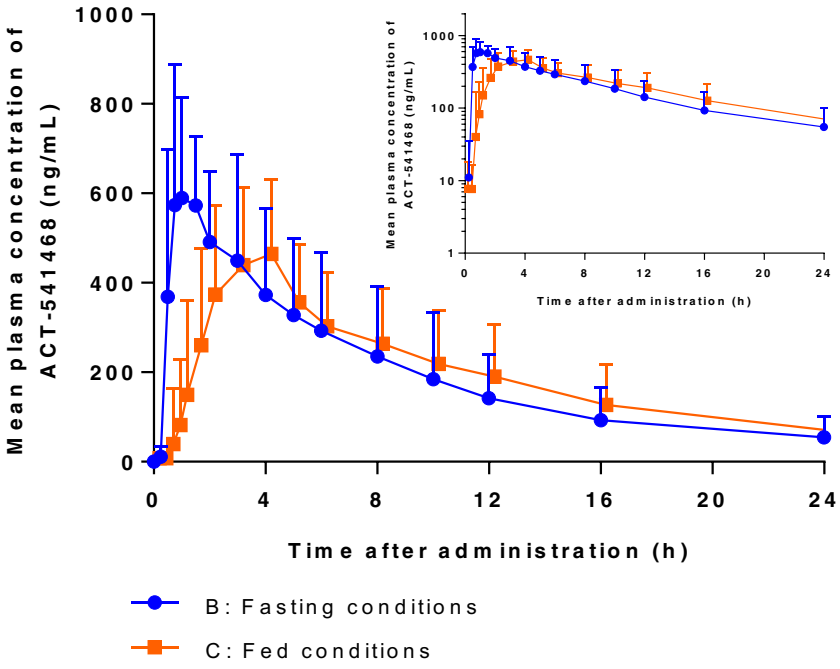

Fig. 4 Arithmetic mean (± SD) plasma concentration–time profile of ACT-541468 after administration of ACT-541468 under fasting and fed conditions ($n = 20$) on a linear and semi-logarithmic scale

CYP3A4 inhibitor may have led to an AUC exceeding that of the highest dose of 200 mg investigated in the single-ascending dose study [14] as preliminary model-based predictions suggested a more than 7-fold increase of ACT-541468 AUC in the presence of a strong inhibitor such as ketoconazole. Even though the study results still allow to conclude that ACT-541468 is a sensitive CYP3A4 substrate, the lacking evaluation of a worst-case DDI scenario has to be considered as study limitation. However, physiology-based PK modeling has recently gained increasing acceptance by the US and European authorities and may be used here to predict changes in drug exposure related to inhibition or induction of drug-metabolizing enzymes [20, 22].

Perpetrator potential of ACT-541468 on CYP3A4 metabolism

In study 2, the well-established CYP3A4 index substrate midazolam was used as interacting treatment and administered at a low dose of 2 mg to ensure an acceptable tolerability when given in combination with ACT-541468 given that both compounds exhibit sedative effects, while still allowing for appropriate evaluation of the PK endpoints [30], and for comparison with other DORAs [23, 29]. Midazolam is mainly metabolized by CYP3A4 in the liver and gut wall [31, 32] to form the major active metabolite 1-OH midazolam. This metabolite is thus a good "biomarker" of any potential modification of CYP3A4 metabolism. The study was designed to evaluate both the CYP3A4 inhibition and induction potential of ACT-541468 in a single study as previously described [33]. Whereas enzyme induction requires a prolonged administration of a perpetrator [34–36], enzyme inhibition/inactivation, even if not maximal, is observed after a single-dose administration of the inhibitor [37, 38]. Thus, in order to adequately assess the CYP3A4 inhibition potential, midazolam was given at the previously reported t_{max} [14] of ACT-541468, i.e., 1 h after ACT-541468 administration, while the induction potential (i.e., net effect of induction/inhibition) was investigated during steady-state conditions [16, 25] of ACT-541468. These were already reached after the second day of administration in line with the previously reported $t_{½}$ of 6.1 h [14] (Fig. S2a).

A lack of perpetrator DDI potential with respect to CYP3A4 has been determined for ACT-541468 given that the 90% CIs of the GMR were all contained in the 0.8–1.25 equivalence boundaries and median t_{max} of midazolam and 1-OH midazolam were not different in the absence or presence of ACT-541468. These results seem contradictory to the ones obtained in vitro where ACT-541468 was shown to be both an inhibitor and inducer of CYP3A4 (data on file). However, taking into consideration a mean plasma C_{max} after single-dose/multiple-dose administration in study 2 of approximately 750 ng/mL (i.e., equivalent to 1.54 μM) and the very high plasma protein binding [13], the levels of unbound ACT-541468 in the present clinical study are at nanomolar level which is far below the required concentration for CYP3A4 inhibition and induction in the liver, i.e., at the micromolar level. Considering a gastric volume < 50 mL under fasting conditions [39] to which are added the 240 mL of water given during administration, the maximum concentration of an oral dose of 25 mg ACT-541468 at the level of the gut would be 0.18 μM, which is also not sufficient to inhibit/induce CYP3A4. The similarity of ACT-541468 plasma exposure observed between Day 1 and Day 5 (Fig. S2b) further supports the lack of effect of ACT-541468 on CYP3A4 in vivo knowing from the study with diltiazem that ACT-541468 PK are modulated by CYP3A4.

With respect to suvorexant, a supra-therapeutic multiple dose of 80 mg increased the AUC of midazolam by 1.5-fold [29] indicating suvorexant as weak inhibitor of CYP3A4.

Interaction of ACT-541468 with food

The effect of food on ACT-541468 PK parameters was evaluated using a high-fat breakfast to ensure a "worst-case scenario" with relation to food intake, i.e., a meal content that induces a maximum effect on the gastrointestinal tract, as per FDA guidance [21] and EMA guidelines [22].

Concomitant administration of a high-fat breakfast had no impact on the extent of ACT-541468 exposure given that $t_{½}$ and AUC remained unchanged. However, a decreased C_{max} and delayed t_{max} has been observed when ACT-541468 was administered together with food. Hence, peak effects of ACT-541468 could principally be decreased and delayed. The underlying mechanisms of such effects were not investigated in the study but decreased C_{max} and delayed t_{max} usually result from a slower gastric emptying rate and/or increased gastric pH induced by food intake [40]. In case of suvorexant, a similar delayed t_{max} (1.5 h) after high-fat breakfast intake has been reported, but C_{max} nor AUC were affected [29].

One limitation of the food effect assessment relates to the use of combined administration of ACT-541468 and midazolam as fasted reference treatment and previously reported midazolam-mediated delay of gastric emptying in mice [41]. However, this is not considered to have introduced major bias given that a much lower dose has been used in our study (2 mg orally, i.e., approximately 0.025 mg/kg) than in mice (25/50 mg/kg intraperitoneally). Moreover, no AEs indicative of delayed gastric emptying were reported.

While food–drug interactions can result in safety concerns when bioavailability is increased (i.e., higher C_{max}), this appears not the case with ACT-541468 given the decrease of C_{max} and the unchanged overall exposure.

Conclusion

In conclusion, these two clinical studies showed that ACT-541468 may be a sensitive CYP3A4 substrate, but is not a perpetrator of CYP3A4-mediated DDIs. Upon concomitant administration of a high-fat breakfast, overall exposure to ACT-541468 was unchanged, while C_{max} was decreased by approximately 25% and t_{max} was delayed by approximately 2 h. Overall, these results support the further development of this new DORA for the treatment of patients suffering from insomnia.

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Compliance with ethical standards

Conflict of interest Actelion Pharmaceuticals Ltd., the predecessor of Idorsia Pharmaceuticals Ltd., provided funding for this clinical study, as owner of ACT-541468. At the time of the study conduct or reporting, M.-L.B., M.U., and J.D. were full-time employees of Actelion Pharmaceuticals Ltd. They are now full-time employees of Idorsia Pharmaceuticals Ltd., the current owner of ACT-541468. A.A. was an employee of Clinical Research Services Kiel GmbH. There are no other relationships or activities that could appear to have influenced the submitted work. Clinical Research Services Kiel GmbH received financial compensation for the clinical conduct.

Statement of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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