Proof of Pharmacology of Org 48775-0, a p38 MAP-kinase inhibitor, in Healthy Volunteers

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Abstract

Aim: To investigate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of the highly selective oral p38α/β MAP-kinase inhibitor Org 48775-0, a first-in-human study was conducted. Methods: In the SAD study part, an oral dose of Org 48775-0 ranging from 0.3 mg to 600 mg was evaluated in healthy males. In the MAD study part, dose levels of 30, 70 and 150 mg were evaluated with dosing for six consecutive days, twice daily. Both studies were performed in a double-blind, randomized, placebo-controlled, cross-over fashion and evaluated pharmacokinetics (PK), pharmacodynamics (PD; inhibition of LPS-induced TNFα release) and routine clinical and laboratory data. Moreover, the effect of a standardized fat meal on PK and PD parameters of Org 48775-0 was evaluated, and PK and PD parameters of Org 48775-0 were compared between healthy males and postmenopausal females. Results: All adverse events observed in the SAD and MAD cohorts were mild, transient and completely reversible without medical intervention. Pharmacokinetics were linear up to single dose s of 400 mg. Org 48775-0 doses equal to and greater than 30 mg significantly inhibited LPS-induced TNFα release (42.3% increase in inhibition, 95% CI=-65.2; -4.3) compared to placebo. In the MAD study, Org 48775-0 treatment inhibited the LPS-induced TNFα release during the entire steady state period. Levels of inhibition amounted 30-75% for 30 mg, 53-80% for 70 mg, and 77-92% for 150 mg Org 48775-0. Conclusion: This study demonstrates that Org 48775-0 has the capacity to significantly inhibit MAP-kinase activity in humans, without raising safety concerns.

Introduction

For more than 25 years, p38 mitogen activated protein kinase (p38 MAP-kinase) represents a pharmacological target in various autoimmune diseases such as rheumatoid arthritis, psoriasis and Crohn's.[1–4] These autoimmune conditions require alternative therapeutic approaches (either as mono-therapy or as add-on therapy combined with drugs like methotrexate, prednisone, and biologicals) to overcome problems such as side effects and the development of drug resistance.[5] p38 MAP-kinase inhibition has been claimed as a promising therapeutic approach for the chronic inflammatory component of neurodegenerative diseases, such as Alzheimer's disease.[6] Activated p38 MAP-kinase activates downstream transcription factors, ultimately resulting in the release of different pro-inflammatory cytokines such as TNFα, IL-1β and IL-6.[7] The MAP-kinase inflammatory pathway can be activated by different receptors including Toll-Like receptors (TLR), cytokine receptors and G-protein-coupled receptors by a variety of stimuli such as bacterial components, superantigen, cytokines, amyloid β-induced cell dysfunction, and heat and osmotic shock.[6, 8] Four subtypes of p38 MAP-kinase have been described (α, β, y, δ), of which p38α is the most relevant subtype involved in the process of inflammation.[9] p38α is expressed in almost all cell types, including inflammatory cells such as monocytes and lymphocytes.[10]*In vitro* inhibition of p38α MAP-kinase decreases the release of

pro-inflammatory cytokines after stimulation with lipopolysaccharide (LPS), a bacterial endotoxin activating the TLR4 pathway, demonstrating the potential therapeutic benefit of inhibition of p38α MAP-kinase in inflammatory conditions.[11, 12] Different p38 MAP-kinase inhibitors have been under preclinical or clinical development, but were halted due to safety concerns or lack of efficacy.[13] Drug development strategies aiming for increased selectivity and potency resulted in the development of the imidazole derivatives, competitive inhibitors at the ATP-binding site of the kinase. Modifications on the imidazole scaffold yielded a large number of potent p38α MAP kinase inhibitors.[14] Recent reviews describe p38 MAP-kinase as potential therapeutic target for inflammatory conditions as asthma.[15]

The synthetic compound Org 48775-0 is a potent and highly selective oral p38α/β MAP-kinase inhibitor (Figure 1). On a panel of more than 100 human kinases, Org 48775-0 was shown to selectively inhibit p38α and β kinases. *In vitro* data demonstrated that Org 48775-0 potently reduces the activation of the p38α signaling pathway in LPS-stimulated immune cells. In mouse models of acute and chronic inflammation a strong, dose-dependent effect of Org 48775-0 was shown. Org 48775-0 reduced LPS-induced TNFα production in mice and inhibited disease progression in collagen-induced arthritic mice. Org 48762-0, a closely related compound, potently inhibited p38a kinase activity and bone damage in a murine model of collagen-induced arthritis.[16]

In this first-in-human single ascending dosing (SAD) and multiple ascending dosing (MAD) study, we investigated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of Org 48775-0 in a in healthy volunteers. In addition, the effect of food on the PK/PD characteristics of Org 48775-0 was evaluated, and potential differences in drug effect between genders were studied. PK and PD parameters of Org 48775-0 were compared between healthy males and postmenopausal females: the prevalence of autoimmune diseases is higher in females than in males [17–19], and gender differences were observed for Org48775-0 pharmacokinetics in preclinical experiments. To account for a potential food effect on drug absorption, as may happen after meals high in fat and calorie content [20], the effect of a standardized fat meal on the PK and PD of Org 48775-0 was evaluated.

Methods

Subjects

Male volunteers between 18 to 65 years of age and an BMI between 18 and 30 kg/m^2, determined to be healthy based on a full medical screening, were included in a first-in-human double-blind, randomized, placebo controlled, cross-over SAD (N=23) and MAD study (N=30) conducted at the Centre for Human Drug Research in Leiden, The Netherlands. Subjects were not allowed to use any medication (except for occasional use of paracetamol or non-steroidal topical medication). Subjects fasted from 10 hours before until 4 hours after each dose administration. Three subjects participated in a preceding exploratory study that guided blood sampling schemes for subsequent SAD cohorts. Furthermore, healthy males were enrolled in an additional food effect cohort (N=6). In addition, a group of healthy postmenopausal females (N=8) between 45 to 65 years of age and a body mass index between 18 and 30 kg/m^2 was included to evaluate potential gender differences in drug effect. All volunteers provided written informed consent before study participation. The studies were approved by the Medical Ethics Review Committee of Leiden University Medical Center (EudraCT number 2007-001993-10) and carried out in accordance with the Declaration of Helsinki. Figure 2 provides a schematic overview of the study design.

*Study design and treatment***Single ascending dosing**

In the SAD study, 23 healthy males received an oral dose administration of Org 48775-0 in a double-blind, randomized, placebo controlled, cross-over fashion. Org 48775-0 was administered orally as a solution in 2x100 mL of orange juice under fasting conditions. Prior to the study, an exploratory study was performed wherein 0.15 mg Org 48775-0 and 0.30 mg Org 48775-0 was administered to 2 volunteers and 1 volunteer respectively, to account for large inter-species differences observed in preclinical pharmacokinetic studies. Using the obtained pharmacokinetic data from this exploratory study, a starting dose of 0.3 mg Org 48775-0 for the SAD study part was determined, followed by 9 consecutive dose levels of Org 48775-0. Subjects were

treated with placebo and maximally 3 ascending doses of Org 48775-0. Each dose was administered 2 weeks after the previous dose to ensure a wash-out period of at least five times $t_{1/2}$. The follow-up visit took place approximately 14 days after administration of the last dose. After each dose administration pharmacokinetic, pharmacodynamic (inhibition of LPS-induced TNFα release), and routine clinical and laboratory data were evaluated and a decision was made on further dose escalation.

Evaluation of food effect The effect of a standardized fat meal opposed to no meal (fasting state) was evaluated in 6 healthy male volunteers who received 100 mg Org 48775-0 in the SAD study. In one occasion, 100 mg Org 48775-0 was administered orally as a suspension in 2x100 mL of orange juice under fasting conditions and in the second occasion (two weeks later), the drug was administered 30 minutes after intake of the standardized meal. The energy content in one standardized fat meal was estimated to be 185 kcal protein, 284 kcal carbohydrates and 482 kcal lipids, which is in accordance with FDA guidelines for food interaction studies (Guidance for Industry; food effect bioavailability and fed bioequivalence studies; 150 kcal protein, 250 kcal carbohydrates, and 500-600 kcal fat). During the second occasion, the subjects started fasting 10 hours prior to drug administration until 4 hours after dosing. The clock-time of drug administration and study-related assessments was comparable for both occasions.

Evaluation of gender differences Potential gender differences in PK and PD characteristics of Org 48775-0 were evaluated after administration of a single dose of 100 mg, in 8 healthy postmenopausal females and 11 healthy males taking part in the SAD study.

Multiple ascending dosing

The effect of repeated oral doses of Org 48775-0 was evaluated in a randomized, double blind, placebo-controlled study in 30 healthy male volunteers. Subjects were treated twice daily for 6 days with Org 48775-0. Three dose levels (30 mg, 70 mg and 150 mg Org 48775-0) were selected (suspended in 2x100 mL of orange juice), based on the results of the SAD study performed in healthy volunteers. Each cohort consisted of 8 subjects that received active treatment and 2 that received placebo. Dose escalation only took place after completion of the safety evaluation of the previous dose level.

Safety assessments

All subjects included were confined for 48-72 hours after drug administration. Recordings of adverse events, standard laboratory parameters (hematology, biochemistry and urinalysis), physical examination, vital signs, temperature and electronic 12 lead electrocardiograms (ECG) measurements (Cardioperfect, Welch Allyn, Rijswijk, The Netherlands) were performed at regular time points before and after dosing. In addition, from 30 minutes before dosing until 12 hours after dosing a continuous 1-lead ECG recording was performed (Nihon Kohden, Tokyo, Japan).

Pharmacokinetic assessments

Since literature suggests a diurnal variation in LPS-induced TNFα release [21], timing of blood sampling was strictly controlled in our study. Clock times for drug administration were standardized within and between study cohorts. Org 48775-0 serum concentrations were determined by at Schering-Plough using a liquid-chromatographic assay with mass spectrometric detection (LC-MS) after solid phase extraction, using a lower limit of quantitation (LLOQ) of 0.0250 ng/mL. Pharmacokinetic parameters of Org 48775-0 were determined based on the individual plasma concentration-time data and included peak plasma concentration (C_{max}), time to reach peak plasma concentration (t_{max}), area under the plasma concentration-time curve extrapolated to infinity ($AUC_{[?]}$), elimination half-life ($t_{\frac{1}{2}}$), and apparent clearance (CL/F).

Pharmacodynamic assessments

Venous blood samples, drawn pre-dose and at regular time points after dosing, were diluted 1:1 with RPMI 1640 medium containing 25 mM Hepes and L-Glutamine (all BioWhittaker). The samples were incubated for 24 hours at 37°C (under 95% O_2/5% CO_2) with a fixed LPS concentration of 5 ng/mL. LPS-induced TNFα levels were measured in the supernantant using a solid-phase, two site chemiluminescent enzyme immuno-

metric assay method (Immulite TNF-α) on a semi-automated analyzer (Immulite 1000, Siemens Medical Solutions Diagnostics B.V.). Blood samples collected before study drug administration were incubated with a concentration range of Org 48775-0 (0.01 to 60 μM) before start of the LPS incubation in to obtain an*in vitro* concentration response curve. LPS challenges were conducted by Good Biomarker Sciences (Leiden, The Netherlands)

Statistical analysis

Pharmacokinetic parameters of Org 48775-0, C_{max}, t_{max}, $AUC_{[?]}$, $t_{\frac{1}{2}}$ and CL/F were determined based on the individual plasma concentration-time data, using ANOVA software. Descriptive pharmacokinetic statistical analysis was only performed if at least 2/3 of the concentrations by time point were greater than or equal to the LLOQ.

The *ex vivo* inhibition of TNFα release after study drug administration was analyzed per cohort, with a mixed model analysis of variance (using SAS PROC MIXED). Data were log-transformed before analysis and treatment effect was reported as active versus placebo, as fasted versus fed for the food interaction study, and as male versus female for the gender effect study. The *in vitro* inhibition of TNFα release was modeled with an inhibitory effect sigmoid Emax model (using SAS PROC LIN; WinNonlin Nonlinear Estimation, software version 5.0, Pharsight, Mountain View, California, USA).**Results**

Subject characteristics

The baseline characteristics of the subjects enrolled in the SAD study (N=23), MAD study (N=30) and food cohort (N=6) and female cohort (N=8) are summarized in Table 1.

Safety assessments

Single ascending dosing Single dose administration up to 600 mg Org 48775-0 in healthy male volunteers did not result in any clinically significant changes in vital signs, ECG-parameters and laboratory parameters. In particular, there were no indications for any change in liver enzymes, signs of bone marrow depression (anemia, thrombocytopenia or leucopenia), or infections (leukocytemia). All adverse events were mild, transient and completely reversible without medical intervention. Six adverse events after dosing with Org 48775-0 were considered during the trial to be possibly related to the study medication. The adverse events consisted of dizziness and headache, one case of diarrhoea and a sterile, catheter-related phlebitis was noted once. Only a few mild adverse events of transient nature were reported in the food and gender interaction study, considered to be unlikely related to study drug administration. Also, none of the participants showed clinically significant changes in vital signs, ECG-parameters or laboratory parameters.

Multiple ascending dosing

Multiple dose administration in healthy male volunteers was not associated with any clinically significant changes in vital signs, ECG-recordings or laboratory parameters. A trend towards elevation was observed in the liver enzyme alanine aminotransferase (ALAT) in several subjects across all dose levels. The increases were completely resolved at the follow up visit. No statistical differences in liver enzyme levels could be observed between any Org 48775-0 treatment and placebo. All adverse events reported in this study were considered mild in severity. Forty-three adverse events after dosing with Org 48775-0 were considered during the trial to be possibly related to the study medication. The main reported adverse events were somnolence, dizziness, headache and nasopharyngitis.

Pharmacokinetics

Single ascending dosing The main pharmacokinetic parameters are summarized in Table 2. The first administration of the low doses showed that Org 48775-0 was rapidly absorbed and eliminated (see Figure 3A). Based on these results a rational sampling scheme was designed for the remainder of the study. From the 100 mg dose upward, a second peak at approximately 4 hrs after dosing was observed. The pharmacokinetics were linear up to a dose of 400 mg and after the 600 mg dose a lower than expected increase in C_{max} and AUC was noted.

Evaluation of food effectT_{max} of Org 48775-0 in fasted condition was 1.6 ± 0.4 hours and in fed condition 3.3 ± 1.0 hours. The $T_{1/2}$ was 12.6 ± 7.1 hours in fasted condition and 9.5 ± 0.6 hours in fed condition. Dosing of 100 mg Org 48775-0 in the fasted condition resulted in C_{max} of 4490 ±949 ng/mL compared to the fed condition (3810 ± 967 ng/mL). With respect to the AUC no relevant differences were observed between fasted (32900 ± 8060 ng/mL) and fed condition (33300 ± 7430 ng/mL) (Figure 3C).

Evaluation of gender differences The pharmacokinetic behavior of Org 48775-0 for females was comparable to males (Figure 3D). $T_{1/2}$ in females was 15.1 ± 6.3 hours and in males 11.4 ± 5.8 hours. The C_{max} in females was 5390 ± 2140 and in males 4474 ± 891 ng/mL. The main pharmacokinetic parameters are summarized in Table 2.

Multiple ascending dosing The pharmacokinetic results showed no accumulation of Org 48775-0 plasma levels after treatment with 30, 70 or 150 mg (see Figure 3B). Steady state concentrations were reached on Day 2 after twice daily dosing of 30-150 mg. Based on the dose-normalized C_{max} values after multiple dose administration, the absorption rate approached its maximum: C_{max} increased less than dose-proportional. This is confirmed by the prolonged T_{max} at 150 mg bid. However, the AUC increased dose-proportionally in the dose range studied. Steady state was reached after 24 hours for all three dose levels.

Pharmacodynamics

Single ascending dosing study

Administration of 0.3, 1, 3 or 10 mg Org 48775-0 did not result in statistically significant differences in LPS-induced TNFα release versus placebo (Table 3; p>0.05). The observed variability in LPS-induced TNFα release over time was in the range of 25-55% (not shown). However, doses equal to and greater than 30 mg Org 48775-0 significantly inhibited LPS-induced TNFα release compared to placebo (Figure 4A, Table 3: 42.3% increase in inhibition versus placebo, 95% CI -65.2; -4.3, p=0.04; maximal inhibition 63% ± 7% at T=1 hour). Maximal inhibition was observed at dose levels exceeding 100 mg (Figure 4A, maximal inhibition >85% at T=1 hour). Doses of 400 mg and 600 mg did not induce an inhibition of TNFα release that was stronger or longer-lasting compared to 200 mg (Figure 4A). Maximal inhibition of the TNFα release was observed in the first 4 hours after dosing.

The *in vitro* Org 48775-0 effect, based on concentration-inhibition curves generated for each study participant in a pre-dose blood sample, very well predicted the *ex vivo* effect of the compound. Figure 5 shows an example of a representative study participant, who received a single dose of 200 mg Org 48775-0. In this subject, the LPS-induced TNFα release was approximately 500 pg/mL at baseline, declining to approximately 100 pg/mL 1 hour after Org 48775-0 administration, after which the TNFα response slowly returned to baseline level over time. This *ex vivo* drug effect mirrored the*in vitro* drug effect at drug concentrations corresponding to the observed serum drug concentrations after oral administration.

Evaluation of food effect There was no significant difference in the LPS-induced TNFα release at baseline between the fasted (361.0 ± 167.1 pg/mL) and fed (290.2± 81.2 pg/mL) condition. No statistically significant effect of food intake was observed on LPS-induced TNFα release after oral administration of 100 mg Org 48755-0 (Figure 4C). In the fasted condition the maximal inhibition of the LPS-induced TNFα release was 80 ± 6% at T=1 hour, in fed condition 73 ± 8%.

Evaluation of gender differences There was no significant difference in the LPS-induced TNFα release at baseline between females (305.3 ± 55.2 pg/mL) and males (436.5± 242.5 pg/mL). No statistically significant difference in maximal inhibition of the LPS-induced TNFα release was observed between males and post-menopausal females (Figure 4D). The inhibition by Org 48755-0 was 83.9% ± 6.2% for males and 78.9% ± 4.3% for females (p=0.31, 95% CI -44.2; 21.0 at 1 hr after dosing). The overall inhibition was smaller in the post-menopausal females compared to the males, as demonstrated by a significant difference in the 0-72 hrs inhibition profiles between males and females (p=0.016, 95% CI -49.6; -8.1). Normalized *in vitro*inhibition curves showed a higher mean EC_{50} for Org 48775-0 in females than in males (0.88 µM versus 0.64 µM, not shown).

Multiple ascending dosing Treatment with 30 mg Org 48775-0, 70 mg Org 48775-0 and 150 mg Org 48775-0 significantly reduced the LPS-induced TNFα release (Table 3: p=0.04, p=0.001, p<0.0001, versus placebo). Maximal inhibition of the LPS-induced TNFα release was reached after administration of the first Org 48775-0 dose (Figure 4B: 75-85%, dependent on dose). During the entire steady state period the inhibition of the LPS-induced TNFα release remained within a range of approximately 30-75% for 30 mg, 53-80% for 70 mg, and 77-92% for 150 mg Org 48775-0. TNFα release returned to baseline levels within 3 days after the final administration of Org 48775-0. There was a possible rebound effect observed for the TNFα release after the 30 mg Org 48775-0 dose, although this effect was not observed for 70 and 150 mg Org 48775-0 (Figure 4B).

Discussion

In this first-in-human single ascending dosing (SAD) and multiple ascending dosing (MAD) study, we investigated the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of Org 48775-0 in healthy volunteers. This study demonstrates that Org 48775-0 has the capacity to significantly inhibit MAP-kinase activity in humans, without raising safety concerns. Single and multiple dose administration of Org 48775-0 to healthy volunteers did not result in any clinical significant changes in vital signs, ECG-parameters and laboratory parameters. There were no indications for any change in liver enzymes, signs of bone marrow depression, or infections. All adverse events were mild, transient and completely reversible without medical intervention. Special attention was paid to ALAT since increases in the level of this transaminase are most directly associated with hepatocyte injury, and the development of other MAP-kinase inhibitors was terminated due to hepatic safety issues.[13, 22] Org 48775-0 was rapidly absorbed and eliminated. Pharmacokinetics were linear up to a dose of 400 mg.

Results further indicate that Org 48775-0 doses equal to and greater than 30 mg significantly inhibited LPS-induced TNFα release, compared to placebo. Maximal drug effect was observed at a dose level of 200 mg, with an inhibition of 87% lasting from 1-4 hours after drug administration, which is in line with the pharmacokinetic behaviour of Org 48775-0. Doses of 400 mg and 600 mg did not induce a stronger or longer-lasting inhibition of TNFα release compared to 200 mg. The*in vitro* Org 48775-0 effect, based on concentration-inhibition curves generated for each study participant in a pre-dose blood sample, very well predicted the *ex vivo* effect of the compound. After multiple dosing, Org 48775-0 treatment inhibited the LPS-induced TNFα release during the entire steady state period. Levels of inhibition amounted 30-75% for 30 mg, 53-80% for 70 mg, and 77-92% for 150 mg Org 48775-0. TNFα release returned to baseline levels within 3 days after the final administration of Org 48775-0.

In addition to the evaluation of the effects of single and multiple doses of Org-48775-0 in healthy males, the effect of food on the PK/PD characteristics of Org 48775-0 was evaluated, and potential differences in drug effect between genders were studied. In line with preclinical data, food intake resulted in an altered pharmacokinetic profile. T_{max} was increased (3.3 versus 1.6 hours) and C_{max} decreased (3810 versus 4490 ng/mL), without a relevant effect on AUC. The pharmacokinetic behavior of Org 48775-0 for females was comparable to males, with a slightly longer $T_{1/2}$ and higher C_{max}. Food intake did not have a significant effect on Org 48775-0 activity, indicating that differences in plasma lipid levels did not impact TNFα release after LPS stimulation in our study. Previous studies reported a potential dampening effect of lipids on LPS-driven inflammation due to a neutralization of the trigger, potentially via lipoprotein binding.[23, 24].

Literature suggests a potential gender difference in cytokine release after stimulation with LPS, male donors having a higher cytokine release than female donors.[23,24] Although our data do not show a significant difference in LPS response between postmenopausal females and males at baseline (possibly due to the relatively small sample size), the TNFα response in males was higher (436 pg/mL versus 305 pg/mL), which is in line with these reports. The maximal inhibition of the LPS-induced TNFα release by Org 48775-0 was comparable between males and post-menopausal females. However, the overall inhibition was smaller in the post-menopausal females compared to the males, as demonstrated by a significant difference in the 0-72 hrs inhibition profiles. This was in line with normalized *in vitro* inhibition curves, showing a higher EC_{50} for Org 48775-0 in females than in males (0.88 μM versus 0.64 μM).

In summary, this study demonstrates that Org 48775-0 administration did not raise safety concerns in healthy human volunteers. A whole blood-based biomarker, LPS-induced TNFα release, was used for evaluation of the pharmacodynamic activity of the investigational compound, both *in vitro* and *ex vivo* . This biomarker showed that Org 48775-0 has the capacity to significantly inhibit MAP-kinase activity in humans, and that the compound exerted its maximal effect at concentrations exceeding 5000 ng/mL, occurring at dose levels of 100 mg and higher. Based on the pharmacodynamic activity of Org 48775-0 *ex vivo* , the optimal dose and regimen for a phase 1B/2 study in the target population can be selected.

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Conflict of interest statement

U. Nässander, R. Nelissen and P.A.M. Peeters were employees of Organon (Oss, The Netherlands) at the time of this research.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1: Baseline characteristics

Mean ± SD (min - max)	SAD (n=23)	Food interaction (n=6)	Female cohort (n=8)	MAD (n=30)
Age (yrs)	35 ± 15 (18 – 64)	23 ± 4 (20 – 31)	58 ± 5 (49 – 63)	33 ± 17 (18 – 64)
Height (m)	1.81 ± 0.06 (1.65 – 1.92)	1.84 ± 0.04 (1.79 – 1.90)	1.67 ± 0.10 (1.47 - 1.76)	1.82 ± 0.07 (1.63 – 1.92)
Weight (kg)	76.3 ± 8.0 (60.7 – 90.0)	76.4 ± 12.9 (60.5 – 95.8)	69.0 ± 16.3 (47.5 - 92.0)	77.2 ± 8.4 (60.5 – 96.4)
BMI (kg/m^2)	23.5 ± 2.8 (19.4 – 28.4)	22.6 ± 3.5 (18.1 – 27.7)	24.7 ± 4.0 (18.1 - 30.0)	23.4 ± 2.6 (18.9 – 29.2)

BMI: body mass index

Table 2: Pharmacokinetic characteristics

Dose (mg)	**t_{half} (h) ± SD**	**C_{max} (ng/mL) ± SD**	**T_{max} (h) ± SD**	**$AUC_{(0\text{-}inf)}$ (ng/mL) ± SD**
SAD				
0.3 mg (n=5)*	7.4 ± 3.1	15.3 ± 2.7	1.4 ± 1.1	117 ± 13.3
1 mg (n=4)	7.1 ± 0.9	43.7 ± 8.8	1.5 ± 0.4	351 ± 61.1
3 mg (n=4)	16.2 ± 8.6	136 ± 53.7	2.3 ± 1.2	1060 ± 307
10 mg (n=3)	9.0 ± 2.0	413 ± 221	0.7 ± 0.3	2660 ± 1830
30 mg (n=4)	8.9 ± 2.2	1520 ± 68.3	1.0 ± 0.0	11600 ± 1740
60 mg (n=6)	9.5 ± 0.7	2700 ± 4.0	0.9 ± 0.6	16100 ± 3840
100 mg (n=5)	10.0 ± 4.1	5110 ± 744	1.3 ± 1.0	35700 ± 6190
200 mg (n=5)	10.9 ± 1.6	6760 ± 1750	1.0 ± 0.4	43500 ± 14200
400 mg (n=6)	9.3 ± 1.0	12600 ± 3430	1.5 ± 0.8	85200 ± 16300
600 mg (n=6)	14.6 ± 12.7	14600 ± 4480	1.3 ± 0.6	96700 ± 17300
Food effect				
100 mg, fasted (n=6)	12.6 ± 7.1	4490 ±949	1.6 ± 0.4	32900 ± 8060
100 mg, fed (n=6)	9.5 ± 0.6	3810 ± 967	3.3 ± 1.0	33300 ± 7430
Gender differences				
100 mg, females (n=6)	15.1 ± 6.3	5390 ± 2140	1.3 ± 0.7	33100 ± 11800
100 mg, males (n=11) **	11.4 ± 5.8	4774 ± 891	1.5 ± 0.7	31129 ± 11474
MAD				
30 mg (n=8)	8.9 ± 1.8	1680 ± 380	1.0 ± 0.5	8000 ± 2060
70 mg (n=8)	13.6 ± 6.6	3000 ± 327	0.8 ± 0.4	14000 ± 1690
150 mg (n=8)	11.6 ± 2.6	5810 ± 1630	1.8 ± 0.6	33600 ± 12100

** Including 1 subject from exploratory study*

*** 5 SAD participants plus 6 food cohort participants (fed condition)*

Table 3: Point estimate and 95% confidence interval of difference in LPS-induced TNFα release (active versus placebo)

Treatment	**Point estimate (%) (95% CI)**	**P-value**
SAD		
0.3 mg	-7 (-43.1; +51.9)	0.71
1 mg	-15.4 (-45.7; +31.8)	0.37
3 mg	-36.9 (-62.2; +5.3)	0.07
10 mg	-23.5 (-48.4; +13.5)	0.12
30 mg	-42.3 (-65.2; -4.3)	0.04
60 mg	-46.2 (-60.3; -27.0)	0.002
100 mg	-72.4 (-81.1; -59.5)	0.002
200 mg	-48.6 (-65.3; -23.9)	0.006
400 mg	-52.9 (-60.6; -43.6)	<0.0001
600 mg	-63.4 (-69.4; -56.1)	<0.0001
MAD		
30 mg BID	-29.8 (-49.8; -2.0)	0.04
70 mg BID	-46.3 (-61.7; -24.8)	0.001
150 mg BID	-72.8 (-80.5; -62.1)	<0.0001

Figure legends:

Figure 1: Chemical structure of Org 48775-0; 4,6-bis(4-fluorophenyl)-5-(4-pyridinyl)-2H-pyrazolo[3,4-b]pyridine-2-propanol.

Figure 2: Schematic overview of the study design

All study parts included male volunteers only, except for the female cohort; PL=placebo.

Figure 3: Pharmacokinetic profile of Org 48775-0

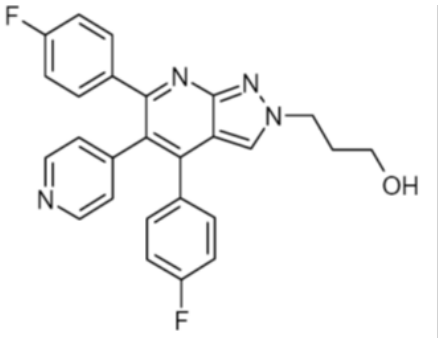
Pharmacokinetic profile of Org 48775-0 per dose average (on y-axis) over time (on x-axis), with SD as error bars. **Panel A** : SAD; 0.3 mg, 1 mg and 3 mg Org 48775-0 are not shown; triangle closed: 10 mg; triangle open: 30 mg; diamond closed: 60 mg; diamond open: 100 mg; triangle down closed: 200 mg; triangle down open: 400 mg; plus: 600 mg;**Panel B** : MAD; circle closed: 30 mg (n=8); circle open: 70 mg (n=8); plus: 150 mg (n=8). **Panel C** : Food effect; circle closed: 100 mg in fasted condition (n=6); circle open: 100 mg in fed condition (n=6). **Panel D** : Gender differences up to 24 hours; circle closed: 100 mg in females (n=6); circle open: 100 mg in males (n=11).

Figure 4: *Ex vivo* inhibition of LPS-induced TNFα release by Org 48775-0

Ex vivo inhibition of LPS-induced TNFα release average per Org 48775-0 dose (on y-axis) over time (on x-axis) with SD as error bars.**Panel A** : SAD; circle closed: placebo; circle open: 0.3 mg; square closed: 1 mg; square open: 3 mg; triangle closed: 10 mg; triangle open: 30 mg; diamond closed: 60 mg; diamond open: 100 mg; triangle down closed: 200 mg; triangle down open: 400 mg; plus: 600 mg. **Panel B** : MAD; circle closed: placebo (n=6); circle open: 30 mg (n=8); square closed: 70 mg (n=8); square open: 150 mg (n=8). **Panel C** : Food effect; circle closed: 100 mg in fasted condition (n=6); circle open: 100 mg in fed condition (n=6). **Panel D** : Gender differences; circle closed: 100 mg in females (n=6); circle open: 100 mg in males (n=11).

Figure 5: *In vitro* versus *ex vivo* Org 48775-0 effect

LPS-induced TNF alpha release for a representative subject. *Ex vivo* Org 48775-0 effect (y-axis) versus *in vitro* Org 48775-0 effect (x-axis), linked based on Org 48775-0 concentration (at 0, 1, 4, 7, 10, 24, 48 and 72 hours; black circles). The dotted line indicates the perfect relationship (*ex vivo* effect = *in vitro*effect).



Study part	Exploratory	SAD	Food	Females	MAD
Design	open	partial 4-way crossover PL-controlled	2-way crossover PL-controlled	PL-controlled	PL-controlled
Groups	0.15 mg (n=2) 0.3 mg (n=1)	0.3, 1, 3 mg and placebo (n=6) 10, 30, 100 mg and placebo (n=5) 60, 100, 200 mg and placebo (n=6) 400, 600 mg and placebo (n=6)	100 mg (n=6) fasted vs fed	100 mg / PL (n=6:2)	30 mg / PL (n=8:2) 70 mg / PL (n=8:2) 150 mg / PL (n=8:2)