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ORIGINAL ARTICLE

Pharmacodynamics, pharmacokinetics and CYP3A4 interaction potential of the selective P2X3 receptor antagonist filapixant: A randomized multiple ascending-dose study in healthy young men

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Aims: We report on investigations exploring the P2X3-receptor antagonist filapixant's effect on taste perception and cough-reflex sensitivity and describe its pharmacokinetics, including its CYP3A4-interaction potential.

Methods: In a randomized, placebo-controlled, double-blind study, 3 × 12 healthy men (18-45 years) were assigned (3:1) to filapixant (20, 80 or 250 mg by mouth) or placebo twice daily over 2 weeks. A single dose of midazolam (1 mg), a CYP3A4 substrate, was administered with and without filapixant. Assessments included a taste-strips test, a taste questionnaire, cough challenge with adenosine triphosphate, adverse event reports and standard safety assessments.

Results: Taste disturbances were observed mainly in the 250-mg group: six of nine participants (67%) in this group reported hypo- or dysgeusia in the questionnaire; eight participants (89%) reported taste-related adverse events. Five participants (56%) had a decrease in overall taste-strips-test scores ≥2 points (point estimate −1.1 points, 90% confidence interval [−3.3; 1.1]). Cough counts increased with adenosine triphosphate concentration but without major differences between treatments. Filapixant exposure increased proportionally to dose. Co-administration of filapixant had no clinically relevant effect on midazolam pharmacokinetics. Area under the concentration-time curve ratios and 90% confidence intervals were within 80-125%. No serious or severe adverse events were reported.

Conclusions: Overall, filapixant was safe and well tolerated, apart from mild, transient taste disturbances. Such disturbances occurred more frequently than expected based on (in vitro) receptor-selectivity data, suggesting that other factors than P2X3: P2X2/3 selectivity might also play an important role in this context. The

Isabella Gashaw is now with Boehringer Ingelheim.

The authors confirm that the principal investigator for this paper is Dave Singh and that he had direct clinical responsibility for the study participants. The study consisted of two parts: part 1 in healthy volunteers (reported here) and part 2 in patients with refractory chronic cough (reported in a separate publication).[1] Professor Morice was appointed coordinating investigator for both parts.

cough-challenge test showed no clear treatment effect. Filapixant has no clinically relevant CYP3A4 interaction potential.

KEYWORDS

assessment of taste function, cough-reflex sensitivity, purinergic receptors, receptor pharmacology, refractory chronic cough, taste disturbances, taste strips

1 | INTRODUCTION

P2X receptors are trimeric adenosine triphosphate (**ATP**)-gated ion channels. Among the P2X family members, homomeric **P2X3** receptors in particular have been recognized as important mediators of nociception and disorders associated with sensory-nerve-fibre overactivation, including genitourinary, gastrointestinal and respiratory conditions.[2–8]

Thus, P2X3 receptor antagonists offer a promising approach for the management of refractory chronic cough (RCC) and potentially also for other conditions such as endometriosis, osteoarthritis and bladder disorders.[9–16]

However, previous studies with the archetype P2X3 antagonist gefapixant reported highly unpleasant disturbances of taste perception in some patients.[17–20] These disturbances were interpreted as off-target effects likely due to blockade of the P2X2/3 receptor heteromer on gustatory nerve fibres.[15] Thus, assuming that taste nerves in humans, just as in mice, mostly express P2X2/3 heteromers, developing receptor antagonists specific for the P2X3 receptor homomer seemed to be the key to avoiding such effects.[15] However, a recent clinical study with eliapixant (BAY1817080), a P2X3 antagonist of the next generation, showed that even highly selective P2X3 antagonists can induce taste disturbances, albeit in a minority of patients.[21] The overall frequency of taste disturbances was substantially lower in this study than previously observed with gefapixant.

In this paper we report the results of pharmacodynamic and pharmacokinetic (PK) investigations with filapixant (BAY1902607), a potent selective P2X3 receptor antagonist under clinical development as a therapy for RCC and endometriosis. Filapixant is very similar to eliapixant in structure and physicochemical and pharmacological properties. The main molecular difference between the two chemical entities is that the tetrahydrofuran group in the eliapixant molecule is replaced by a methylmorpholine group in the filapixant molecule.[22,23] This results in filapixant having better solubility at low pH than eliapixant and even higher selectivity in vitro for P2X3 over P2X2/3 (~13-20-fold[15] *vs* >100-fold) (unpublished data, Bayer AG, Berlin, Germany). This was expected to lead to an even better tolerability—that is, fewer taste alterations—while the beneficial effect on cough was expected to be the same.

Thus, we focus in this paper on the investigations conducted to study filapixant's potential effect on taste perception in particular and on its effect on cough-reflex sensitivity. These investigations were conducted in an exploratory fashion as part of an early phase 1 study, which was primarily designed to study the safety and tolerability of ascending repeated oral doses of filapixant in healthy volunteers and to investigate its CYP3A4 interaction potential.

What is already known about this subject

- Studies have shown that P2X3 receptor antagonists such as gefapixant, which show little P2X3:P2X2/3 selectivity, frequently cause taste disorders, which are attributed to off-target effects at the P2X2/3 receptor.

What this study adds

- The study showed that highly selective P2X3 receptor antagonists such as filapixant can also induce taste impairments.
- This suggests that factors other than P2X3:P2X2/3 selectivity might play an important role in this context.
- Supplementary investigations are needed to explore the pharmacodynamic properties of filapixant.

Following this phase 1 study, a proof-of-concept study in patients with RCC was conducted under the same protocol. The results of this phase 2a study have been reported in a separate publication.[1] The primary endpoint of the study was the 24-h cough count, which can be considered the gold standard to prove the efficacy of novel cough medications.[21,24,25]

2 | METHODS

2.1 | Study population

This was a phase 1 study in healthy young men. To be eligible, prospective participants had to be between 18 and 45 years of age (inclusive), have a body mass index ≥18 and ≤30 kg/m^2, be nonsmokers (for ≥6 months) and have a smoking history of ≤5 pack years. The concentration of the ATP solution required to induce at least two coughs in the screening inhalational cough-challenge test had to be ≤128 mg/mL. Individuals with a forced expiratory volume in 1 s or forced vital capacity <80% of predicted normal at screening were excluded from participation as were individuals who were not able to taste at least the second-highest concentration of each taste quality in the screening taste-strips test or reported taste disturbances in the taste-disturbance questionnaire

(see Supporting Information Chapter S1 for the complete list of entry criteria and Section 2.3.1 for a description of the cough-challenge test and the methods used to evaluate changes in taste perception).

Women were excluded from participation for safety reasons because no reproductive-toxicology data were available when the study was conducted.

2.2 | Study design and treatments

This was a randomized, placebo-controlled, double-blind, multiple ascending-dose study with three parallel dose groups. Each group included 12 participants. Nine participants per group received the investigational product filapixant (20, 80 or 250 mg twice daily [BID] over 2 weeks) and three participants received placebo. Dosing started with the lowest dose; escalation to the next dose level took place only after thorough review of the safety, tolerability and PK data from the preceding dose level. Population PK modelling and simulation based on single-dose data from the first-in-human study (clinicaltrials.gov identifier: NCT03212586), which indicated that the systemic exposure of filapixant increases proportionally to dose under fasting conditions with a half-life of 12 to 15 h (unpublished data on file, Bayer AG), was used to select the dose steps of interest. The preliminary safety, tolerability and PK data obtained in the first-in-human study of filapixant, in which single doses of up to 1250 mg were tested (to be published separately), supported the repeated administration of filapixant 20-250 mg BID over 2 weeks. The filapixant exposure in the present study was expected to be approximately 50% of the highest dose tested in the first-in-human-study. PK steady state was expected after 2-3 days of treatment, assuming a terminal half-life of 12-15 h.

At the 20-mg level, the typical steady-state plasma trough concentrations of filapixant were expected not to exceed the half-maximal inhibitory concentration (IC_{50}) of the compound measured using an in vitro fluorescence imaging plate reader-based calcium flux assay and a human patch clamp assay (unpublished data on file, Bayer AG). At the 80-mg level, typical plasma trough concentrations covering the IC_{50} but not exceeding the IC_{80} of filapixant were expected, and at the highest dose level, 250 mg, typical plasma concentrations of filapixant covering the IC_{80} over the whole dosing interval were expected. The originally planned 500-mg-dose step aiming at IC_{80} coverage in at least 90% of participants was omitted because plasma concentrations covering the IC_{80} in about 90% of participants were observed already with 250 mg filapixant under nonfasting conditions (unpublished data on file, Bayer AG). IC_{80}, that is, 80% receptor occupancy, is the expected efficacy threshold in patients with RCC.[15]

The study comprised a screening and randomization phase, a 14-day treatment period and a follow-up visit for each participant (Figure 1). Participants were assigned randomly (3:1) to treatment with filapixant or placebo according to a computer-generated randomization list.

Filapixant was given in the form of 10-, 50- and 200-mg immediate-release tablets manufactured by Bayer. A first single dose of filapixant (20, 80 or 250 mg) was given on day 0, from day 1 to day 12, the same dose was administered BID and on day 13 a last single dose was given. The participants assigned to placebo treatment received matching placebo tablets. Additionally, as filapixant was identified in vitro as an inducer of CYP3A4 (unpublished data on file, Bayer AG), a subtherapeutic dose of midazolam (1 mg as oral solution; Midazolam-ratiopharm 2 mg/mL; ratiopharm, Ulm, Germany), a typical probe substrate to elucidate CYP3A4-mediated drug-drug interactions,[26–28] was given alone (day −1) and together with the last dose of filapixant (day 13) to determine the effect of filapixant on its PKs. All study drugs were administered within 30 min after the start of a meal. Administration in the fasted state was not an option with twice-daily administration, that is, in particular not for the evening dose. The study was conducted under double-blind conditions, that is, study personnel and participants were blinded to the treatment. Active drug and placebo tablets (for each strength) were identical in appearance, taste and smell.

The concomitant use of any CYP3A4 inducers, inhibitors and substrates was not allowed from 2 weeks before the first dose until the follow-up visit. Opioids and over-the-counter cough mixtures were not allowed from 1 week or 24 h, respectively, before screening until the follow-up visit to avoid distortion of the cough-challenge test results.

2.3 | Procedures and variables

2.3.1 | Pharmacodynamics

A taste disturbance (dysgeusia) questionnaire-based interview (Supporting Information Chapter S2) and a taste-strips test[29] were used to evaluate the potential effects of filapixant on taste perception. Four taste qualities were tested with taste strips soaked with flavoured solutions at four different concentrations (Burghart Messtechnik GmbH): sweet (sucrose 0.4, 0.2, 0.1 and 0.05 g/mL), sour (citric acid 0.3, 0.165, 0.09 and 0.05 g/mL), salty (sodium chloride 0.25, 0.1, 0.04 and 0.016 g/mL) and bitter (quinine hydrochloride 0.006, 0.0024, 0.0009 and 0.0004 g/mL). Furthermore, taste strips without any flavour were used. Presentation of taste strips started with the lowest concentration of each taste quality, followed by the second lowest concentration and so on. At each concentration level, the four different taste qualities were presented in randomized order. The participants were asked to close their mouths after placement of the strip and move their tongue, if desired (whole-mouth testing). Before and after each strip application, the mouth was rinsed with tap water. Correct identification of the taste quality of all 16 strips (four taste qualities × four intensities) yielded 16 points.

Taste assessments by questionnaire and taste-strips test were supplemented by adverse event reports as part of the safety assessments.

A cough-challenge test with ATP as the challenge agent[30] was used to study the effect of filapixant on cough-reflex sensitivity. The participants inhaled ATP in 0.9% saline through a calibrated nebulizer attached to a dosimeter. The concentration of ATP in saline was doubled step by step, starting with 0.125 mg/mL and ending with the highest concentration tolerated by the participant (maximum 512 mg/mL). Each concentration was inhaled four times, 30 s apart, before

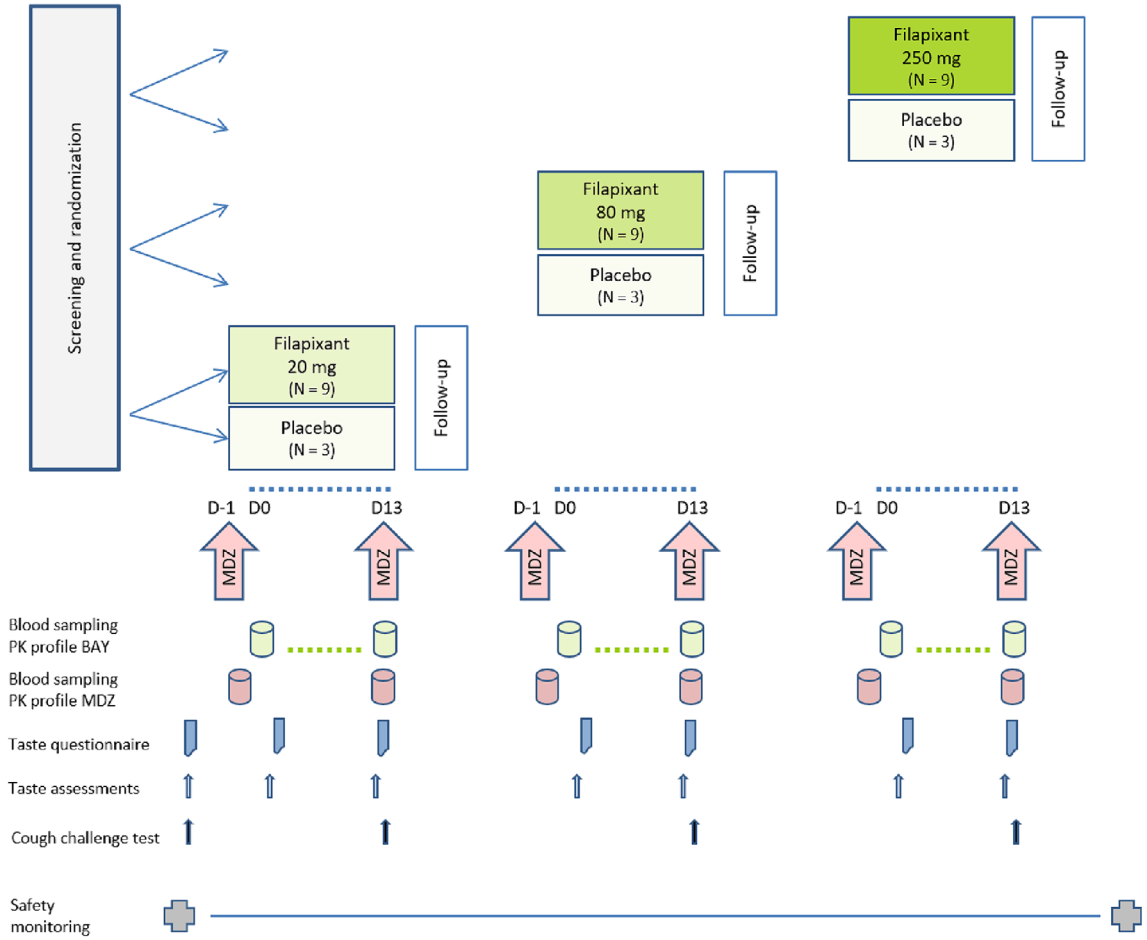

FIGURE 1 Study design. Filapixant or placebo were administered twice daily (from days 1 to 12) and once daily on days 0 and 13. Midazolam was administered as a single dose on days −1 and 13 of each dose step. Escalation to the next higher dose level took place only after review of the safety, tolerability and pharmacokinetic data from the preceding dose level. Screening took place within the 4 weeks before first study drug administration, the end-of-study visit 7 to 21 days after the last dose. Blood samples for midazolam pharmacokinetics were taken pre dose and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 15 and 23.25 h postdose on days −1 and 13. Essentially the same schedule was used on days 0 and 13 for filapixant pharmacokinetics samples. Trough samples were taken at regular intervals during treatment. At day −1, the taste questionnaire was completed before taste assessments, at days 3 and 13 approximately 2 and 3 h, respectively, after the assessments. Taste assessments (taste-strips tests) were carried out before any treatment and 0.5 h before intake of the morning dose at days 3 and 13. Cough-challenge tests were run pre dose and 6 h after the last dose at day 13. Abbreviations: D ..., day number ...; MDZ, midazolam; PK, pharmacokinetic.

escalation to the next level. The participants were instructed to inhale slowly and to cough as much as they felt they needed to. The number of coughs within 15 s of inhalation was counted for each repetition of the test. Based on these counts, the ATP concentrations that induced ≥2 and ≥5 coughs (C2 and C5) were determined. The focus of the study was on (i) the lowest ATP concentration at which the event (≥2 or ≥5 coughs) occurred in any participant, (ii) the lowest ATP concentration at which ≥70% of participants responded with ≥2 or ≥5 coughs and (iii) the ATP concentration at which the highest percentage of participants responded with ≥2 or ≥5 coughs.

All three procedures described above are well-established methods.[31–33] The taste strip test method has been used in previous studies where normative values for a large population have been collected. In addition, the accuracy and reliability of the test have been shown under repetitive use (unpublished data on file, Bayer AG). Matching the cough triggering pathway affected by P2X3 antagonists, we used ATP as the tussive agent in the cough-challenge test. The ATP cough challenge has also been used in prior studies of gefapixant and eliapixant.[19,34–36] The taste disturbance (dysgeusia) questionnaire we used is a slightly modified extract from a questionnaire designed to document the medical history of patients with olfactory disorders.[37,38] The complete medical history form is widely used in German-speaking countries and internationally.

2.3.2 | Pharmacokinetics

Blood samples for PK analyses of filapixant, midazolam and its metabolite 1′-OH-midazolam in plasma were collected at the time points specified in Figure 1. The samples were analysed using validated

analytical methods (details in Supporting Information Chapter S3). Standard noncompartmental PK parameters were calculated for the three analytes using WinNonlin v5.3 (Certara Companies). The primary PK parameters for assessing the CYP3A4-induction potential of filapixant were the area under the concentration-time curve (AUC) and the observed maximum concentration (C_{max}) of midazolam in plasma.

2.3.3 | Safety and tolerability

Spontaneously reported or observed adverse events (AEs) and AEs mentioned on open questioning were documented throughout the study. Other safety assessments included standard clinical laboratory tests, vital signs, electrocardiograms and physical examinations. The forced expiratory volume was monitored before and after the challenge test to check for signs of bronchoconstriction.

Primary variables for the assessment of safety and tolerability were the frequency and severity of treatment-emergent AEs.

2.4 | Analysis

Statistical analyses were conducted using the program package SAS v9.4 (SAS Institute). All analyses were exploratory, therefore no multiplicity adjustments were performed. All participants on placebo were pooled (placebo group). Baseline was defined as the last nonmissing value before the start of treatment. For taste scores, point estimates and parametric 95% confidence intervals (CIs) for changes from baseline were determined for each dose group. No statistical tests were conducted for questionnaire data, taste-related AE reports and cough-challenge data.

For the investigation of drug-drug interactions, an analysis of variance model was used on log-transformed AUC and C_{max} of midazolam as the dependent variables. Treatment with filapixant (yes/no), dose group and their interaction were used as fixed effects, and subject nested within dose group as a random effect. Least squares means differences and 90% CIs were determined for the treatment effects and dose × treatment interactions and subsequently re-transformed to the original scale.

To investigate dose proportionality, analyses of variance (with treatment as single factor) were performed on the log-transformed, dose-normalized AUC(0-12) and Cmax of filapixant (multiple dose). Point estimates and 90% CIs of the treatment effect of each dose were calculated and then re-transformed to the original scale. In addition, the power model[39] was applied to assess the dose proportionality. No formal statistical interim analyses were performed.

2.5 | Sample size considerations

Based on prior experience in similar phase 1 studies, a "standard" sample size of 12 subjects per dose level (three on placebo) was expected to be sufficient to fulfil the primary objectives of this study, that is, to explore the safety of repeated doses of filapixant and to evaluate the CYP3A4-interaction potential of filapixant. A power calculation for exploratory pharmacodynamic measures was neither planned nor performed.

TABLE 1 Participant characteristics (N = 36).

		Placebo (N = 9)	Filapixant 20 mg (N = 9)	Filapixant 80 mg (N = 9)	Filapixant 250 mg (N = 9)
Sex	Male	9 (100.0%)	9 (100.0%)	9 (100.0%)	9 (100.0%)
Race	Asian	1 (11.1%)	0	0	0
	Black/African American	2 (22.2%)	1 (11.1%)	0	0
	White	6 (66.7%)	8 (88.9%)	8 (88.9%)	9 (100.0%)
	Other	0	0	1 (11.1%)	0
Ethnicity	Hispanic or Latino	0	0	1 (11.1%)	0
	Not Hispanic or Latino	8 (88.9%)	8 (88.9%)	7 (77.8%)	9 (100.0%)
	Not reported	1 (11.1%)	1 (11.1%)	1 (11.1%)	0
Age (years)	Mean ± SD	31.0 ± 8.0	26.9 ± 7.6	31.4 ± 6.4	33.2 ± 8.5
	Range	22-45	18-39	20-44	19-43
Body mass index (kg/m^2)	Mean ± SD	25.37 ± 2.84	25.31 ± 2.86	25.42 ± 1.96	25.08 ± 2.15
	Range	22.2-29.6	21.6-29.8	22.6-28.3	20.8-27.7
Smoking history	Never	8 (88.9%)	6 (66.7%)	6 (66.7%)	9 (100.0%)
	Former	1 (11.1%)	3 (33.3%)	3 (33.3%)	0
Other (tobacco/cigar/pipe)	Never	8 (88.9%)	9 (100.0%)	9 (100.0%)	9 (100.0%)
	Former	1 (11.1%)	0	0	0

Abbreviations: N, number of participants; SD, standard deviation.

With regard to the evaluation of the filapixant's drug-drug interaction potential, a sample size of seven subjects per dose level was determined to be sufficient to achieve a half-width of the 90% CI for the ratio AUC $_{\text{midazolam with filapixant}}$/AUC $_{\text{midazolam alone}}$ within the range (0.87; 1.15) with at least 80% probability (assuming a within-subject coefficient of variation of 30%).

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24.[40]

3 | RESULTS

3.1 | Study participants

The study was conducted at one study centre in the UK between May and November 2018.

Of 91 prospective participants screened, 55 men were screening failures or were excluded for other reasons; 36 men were enrolled, randomized and received study medication (Supporting Information Chapter S4, Figure S1). All 36 participants completed the study; no participant was excluded from analysis or re-assigned to another treatment group for analysis. The demographic characteristics were well balanced among the four treatment groups (Table 1).

3.2 | Pharmacodynamics

3.2.1 | Taste assessments

The overall taste-strips-test scores showed signs of a decrease in taste perception from baseline to day 13, mainly in some participants from the 80- and 250-mg groups. In these two groups, decreases in overall scores by ≥2 points from baseline were seen in four and five of nine participants, respectively (44% and 56%) (Figure 2; Supporting Information Chapter S4, Figure S2). In the 20-mg group and the placebo group, in contrast, reductions by ≥2 points were rare (one and two of nine participants, respectively [11% and 22%]). The mean change (± standard deviation) in overall test scores amounted to −1.4 ± 2.6 points (range −5 to +3 points) in the 80-mg group and −1.1 ± 2.8 points (range −6 to +4 points) in the 250-mg group (Supporting Information Chapter S4, Table **S1**). Point estimates and CIs for changes from baseline indicate no statistically significant change in overall test scores in any of the treatment groups (box in Figure 2, Supporting Information Chapter S4, Table S2). The distribution of taste quality-specific scores does not suggest that certain taste qualities were particularly affected (Supporting Information Chapter S4, Table S1 and Figure S3A-D).

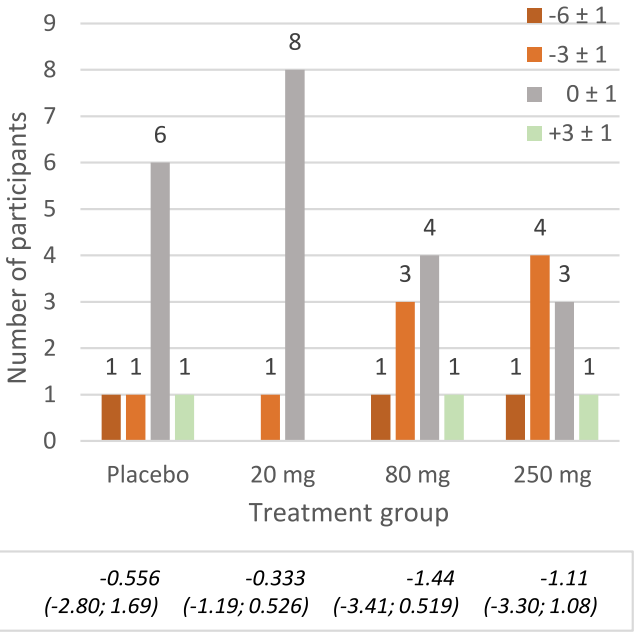

FIGURE 2 Taste-strips test: overall taste-strips-test scores at the end of multiple-dose treatment (changes from baseline). Bars represent the number of participants whose overall taste-strips-test scores changed by the number of points indicated by the colour code. No change from baseline is represented by grey, decreases by different shades of red-orange, increases by different shades of green as specified in the legend. Changes from baseline exceeding −7 or +4 points were not observed. For clarity, scores are aggregated in this graph. Nonaggregated data are shown in Supporting Information Chapter S4, Figure S2. Point estimates (least square means) and 95% confidence intervals for changes from baseline are provided in the box at the bottom of the figure. Overall taste-strips-test scores could range from 0 to 16. They reflect the total number of correct identifications (four taste qualities × four intensities). Each treatment group included nine participants.

In the questionnaire-based interview, none of the participants reported taste disturbances before dosing and only participants from the 250-mg group reported taste disturbances after dosing: In total, six of nine participants (67%) from this group answered "Yes" to the introductory question about taste dysfunctions. Dysgeusia with regard to the taste qualities sweet, salty, sour, bitter and "hot" was described as well as hypogeusia and permanent oral sensations (details in Table 2).

Taste-related AEs—that is, taste disturbances or changes in taste (with or without further specification), metallic, soapy or salty taste—were reported mainly, but not exclusively, by participants from the 250-mg group (Table 2). One participant from the 20-mg group (11%) and one participant (11%) from the 80-mg group also reported taste disturbances. These AEs started at days 5 and 11, respectively, and lasted for 1 or 2 h. Taste disturbances in the 250-mg group, in contrast, which were reported by eight of nine participants (89%), mostly started right at the beginning of treatment and persisted throughout the entire treatment period (details in Supporting Information Chapter S4, Table S3). Some participants

TABLE 2 Taste assessments: individual questionnaire data (A) and taste-related adverse events (B).

	Filapixant										
	20 mg	80 mg	250 mg								
Participant ID[a]	11	21	31	32	33	34	35	36	37	38	39
(A) Taste-disturbance questionnaire											
1. Dysfunction in ability to taste	··	··	·X	·X	·X	··	··	··	XX	X·	XX
2. Dysgeusia had to do especially with the sensation											
Sweet			·X	··	··				··	··	··
Salty			··	·X	··				··	X·	·X
Hot			·X	··	··				··	··	··
Sour			·X		··				··	··	··
Bitter			·X	··	··				··	··	··
None of the above			··	··	·X				XX	··	X·
3. Reduction of taste sensation			·X	·X	·X				·X	··	··
4. Classification of taste reduction											
No change of sensation			··	··	··				··	··	··
Reduction less than during a cold			·X	·X	··				··	··	··
Reduction as during a cold			··	··	·X				··	··	··
Complete loss of sensations of one/some tastes			··	··	··				··	··	··
Complete loss of sensations of all tastes			··	··	··				··	··	··
5. Permanent[b] sensation in the mouth											
Burning sensation	··	··	··	··	··	··	··	··	··	··	··
Sour taste	··	··	··	··	··	··	··	··	··	··	··
Bitter taste	··	··	··	··	X·	··	··	··	··	··	··
Dryness of the mouth	··	··	XX	··	··	··	··	··	··	··	··
Salty taste	··	··	··	X·	··	··	··	··	··	XX	XX
Foreign body sensation	··	··	·X	··	··	··	··	··	··	··	··
(B) Reports of taste-related adverse events	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Taste disturbance NOS	x	x	x[c]		x[d]						
Soapy taste				x							
Chalky taste						x					
Metallic taste								x		x	x
Salty taste										x	
Change in taste									x		

Notes: The numbers of the items in part A of the table refer to the respective questions in the questionnaire (Supporting Information Chapter S2). The question about dysfunction in ability to taste (question 1) was the introductory question. If it was answered in the affirmative, the participant was asked to specify the dysgeusia and the reduction in taste sensation. ··, question answered in the negative at both assessments; ·X or X·, question answered in the negative at day 3 and in the affirmative at day 13 or vice versa; XX, question answered in the affirmative at both assessments. Pre-dose, no taste-related adverse events were reported and none of the questionnaire questions was answered with Yes.

Abbreviations: NOS, no other specification.

[a]From the 20-mg and 80-mg group, only those participants are listed who reported any taste-related adverse events or answered at least one question from the questionnaire with Yes.

[b]'Long-lasting' according to the participant's assessment, that is, lasting for some time after the respective stimulus had disappeared, the food been swallowed, for example, or independent of any stimulus.

[c]The participant described the event as 'intermittent and "waxy" taste'.

[d]The adverse event was described as 'intermittent, would coincide with dosing. Adverse event would start approximately one hour after dosing and then wear off'.

described their taste disturbances as intermittent. The duration of taste-related AEs varied between 6 h and 14 days in this group. All taste-related AEs were mild, did not lead to discontinuation of treatment and had resolved completely by the time of the follow-up visit. None of the participants from the placebo group reported taste-related AEs.

Table 2 shows that the responses to the questionnaire items and reports of taste-related AEs only partially overlap. On the one hand, there were six participants from the 250-mg group who reported taste-related AEs and also answered the question about dysfunctionality in ability to taste in the affirmative. On the other hand, there were two other participants from this group and one participant each from the 20- and 80-mg groups who reported taste-related AEs but responded to the question about dysfunctionality in ability to taste in the negative.

3.2.2 | Cough-challenge test

As expected, the mean total cough count and the number of participants who responded with ≥2 and ≥5 coughs to the ATP challenges generally increased with ATP concentration; however, the variability of the cough responses was high (Supporting Information Chapter S4, Figure S4 and Table S4). In particular, the almost complete absence of ≥5 coughs responses in the 80- and 250-mg groups at baseline and postdose is noteworthy. Overall, the number of participants responding with ≥2 coughs (≥5 coughs) seemed to slightly decrease from baseline to postdose in all treatment groups, including placebo.

There were no major differences between baseline and postdose tests regarding the lowest ATP concentration at which any participant responded with ≥2 coughs, or the ATP concentrations at which >70% of participants responded with ≥2 coughs or the ATP concentration at which the highest percentage of participants responded with ≥2 coughs (Table 3).

3.3 | Pharmacokinetics

3.3.1 | Effects of filapixant co-administration on the PKs of midazolam and 1-OH-midazolam

A summary of the key PK parameters of midazolam and 1-OH-midazolam in plasma obtained after single administration of 1 mg midazolam alone (day −1) and with co-administration of 20, 80 or 250 mg of filapixant BID over approximately 2 weeks (day 13) is presented in Supporting Information Chapter S4, Table S5. Mean midazolam and 1-OH-midazolam plasma concentration-time curves are provided in Supporting Information Chapter S4 (Figure S5A-D and Figure S6A-D).

Geometric least square means and 90% CIs for the ratios midazolam with co-treatment/midazolam alone of AUC and C_{max} for midazolam and 1-OH-midazolam are shown in Table 4. No significant effect of filapixant co-administration on the exposure of midazolam was found. The 90% CIs for the three AUC ratios were well within the established bioequivalence range of 0.80-1.25. The point estimates and 90% CIs for C_{max} ratios indicated a slight increase in midazolam C_{max} when the drug was co-administered with 20 or 80 mg of filapixant. T_{max} and $t_{1/2}$ were similar with and without co-administration of filapixant. 1-OH-midazolam AUC and C_{max} values were slightly higher with co-administration of filapixant than without.

3.3.2 | PKs of filapixant

A summary of the key PK parameters of filapixant in plasma obtained after single administration of filapixant (day 0) and after BID

TABLE 3 ATP cough-challenge test: C2 and C5 analysis.

Treatment group	Assess-ment	Lowest concentration of ATP that induced at least 2 (or 5) coughs[a] in any participant		Lowest concentration of ATP that induced at least (or 5) coughs[a] in >70% of participants		Concentration of ATP that induced at least 2 (or 5) coughs[a] in the largest fraction of participants			
		C2 (mg/mL)	C5 (mg/mL)	C2 (mg/mL)	C5 (mg/mL)	C2[b] (mg/mL)		C5[b] (mg/mL)	
Placebo	Predose	2	2	32	N/O	512[c]	(100%)	256[c]	(50%)
	Postdose[c]	2	16	128	N/O	512[c]	(88%)	512[c]	(25%)
20 mg of filapixant	Predose	1	64	128	N/O	256	(100%)	512	(44%)
	Postdose [c]	4	32	128	N/O	512	(100%)	128	(33%)
80 mg of filapixant	Predose	2	512	512	N/O	512	(90%)	512	(22%)
	Postdose[c]	32	512	512	N/O	512	(90%)	512	(11%)
250 mg of filapixant	Predose	32	128	512	N/O	512[d]	(100%)	128	(11%)
	Postdose[c]	16	128	512	N/O	512[d]	(88%)	128	(11%)

Abbreviations: ATP, adenosine triphosphate; C2, ATP concentration that induced at least two coughs within 15 s of inhalation; C5, ATP concentration that induced at least five coughs within 15 s of inhalation; N/O, not observed in >70% of participants; postdose, 6 h after administration of the last dose of filapixant or placebo and midazolam.

[a]Within 15 s of inhalation.

[b]The ATP concentration is followed (in parentheses) by the percentage of participants who met the respective criterion. Percentages are based on the number of participants exposed to the respective ATP concentration. If the percentage was the same at two or more ATP levels, the lower or lowest concentration is given.

[c]6 h after the last dose at day 13.

[d]N = 8. A few participants terminated the test before having reached the highest ATP concentration.

TABLE 4 Geometric least square means and 90% confidence intervals for the ratio midazolam with filapixant/midazolam alone of AUC and C_{max} for midazolam and 1-OH-midazolam in plasma.

		Midazolam		1-OH-midazolam	
Parameter (unit)	Dose of filapixant (mg)	Geometric LS mean	90% confidence interval	Geometric LS mean	90% confidence interval
AUC (ng·h/L)	20	1.0670	(0.9896; 1.1503)	1.1244	(1.0044; 1.2588)
	80	0.9745	(0.9039; 1.0507)	1.5757	(1.4075; 1.7639)
	250	1.1000	(1.0203; 1.1860)	1.3707	(1.2244; 1.5344)
C_{max} (ng/L)	20	1.2499	(1.0793; 1.4476)	1.4557	(1.1724; 1.8074)
	80	1.2877	(1.1118; 1.4913)	2.3670	(1.9064; 2.9388)
	250	1.0200	(0.8807; 1.1812)	1.3544	(1.0908; 1.6816)

Note: $N = 9$ participants per treatment group.
Abbreviations: AUC, area under the concentration-time curve from zero to infinity; C_{max}, observed maximum concentration in plasma; LS, least square.

TABLE 5 Pharmacokinetic parameters of filapixant in plasma after single and multiple administration of 20, 80 or 250 mg of filapixant.

Parameter (unit)	Filapixant 20 mg N = 9	Filapixant 80 mg N = 9	Filapixant 250 mg N = 9
Single-dose administration (day 0)			
AUC (µg·h/L)	255 (11)	829 (27)	2650 (25)
AUC/D (h/L)	0.0128 (11)	0.0104 (27)	0.0106 (25)
AUC(0-12) (µg·h/L)	176 (5.4)	565 (23)	1660 (25)
AUC(0-12)/D (h/L)	0.00882 (5.4)	0.00707 (23)	0.00665 (25)
C_{max} (µg/L)	29.3 (19)	93.7 (35)	260 (30)
C_{max}/D (1/L)	0.00146 (19)	0.00117 (35)	0.00104 (30)
$t_{1/2}$ (h)	8.98 (32)	8.88 (19)	10.3 (20)
t_{max} (h)	2.00 (1.0-4.0)[a]	1.50 (1.0-2.5)[a]	2.00 (1.0-4.0)[a]
Multiple-dose administration (day 13)[b]			
$AUC(0\text{-}12)_{md}$ (µg·h/L)	261 (17)	847 (30)	3100 (30)
$AUC(0\text{-}12)_{md}$/D (h/L)	0.0131 (17)	0.0106 (30)	0.0124 (30)
$C_{max,md}$ (µg/L)	39.6 (13)	133 (23)	418 (23)
$C_{max,md}$/D (1/L)	0.00198 (13)	0.00166 (23)	0.00167 (23)
$t_{1/2,md}$ (h)	13.4 (20)	12.8 (19)	12.0 (15)
$t_{max,md}$ (h)	1.00 (0.5-3.0)[a]	1.50 (0.5-2.5)[a]	2.00 (0.5-4.0)[a]
CL_{md}/F (L/h)	76.5 (17)	94.5 (30)	80.7 (30)
R_{LIN}	1.02 (16)	1.02 (22)	1.17 (19)
$R_{A\ (AUC)}$	1.48 (18)	1.50 (26)	1.86 (26)
$R_{A\ (Cmax)}$	1.35 (27)	1.42 (29)	1.61 (31)

Note: Data are geometric means followed by geometric coefficients of variation in parentheses unless indicated otherwise.
Abbreviations: AUC, area under the concentration-time curve from zero to infinity after single-dose administration; AUC/D, dose-normalized AUC; AUC(0-12), area under the concentration-time curve from zero to 12 h postdose; AUC(0-12)/D, dose-normalized AUC(0-12); CL_{md}/F, total body clearance of drug calculated after multiple oral administration (apparent oral clearance); C_{max}, observed maximum concentration in plasma; C_{max}/D, dose-normalized C_{max}; CV%, coefficient of variation (%); D, dose; md, the subscript 'md' indicates that the parameter refers to multiple-dose administration; N, number of participants; $R_{A\ (AUC)}$, accumulation ratio calculated as $R_{A\ (AUC)} = AUC(0\text{-}12)_{md}/AUC(0\text{-}12)_{sd}$; $R_{A\ (Cmax)}$, accumulation ratio calculated as $R_{A\ (Cmax)} = C_{max,md}/C_{max,sd}$; R_{LIN}, linearity index of pharmacokinetics after repeated administration of identical doses calculated as $R_{LIN} = AUC(0\text{-}12)_{md}/AUC$; sd, the subscript 'sd' indicates that the parameter refers to single-dose administration; $t_{1/2,md}$, half-life after multiple administration; $t_{max,md}$, time to maximum concentration after multiple administration.
[a]Median followed by minimum and maximum in parentheses.
[b]On day 13, a single dose of filapixant was taken together with a 1-mg dose of midazolam. On days 1 to 12, filapixant was taken twice daily without comedication.

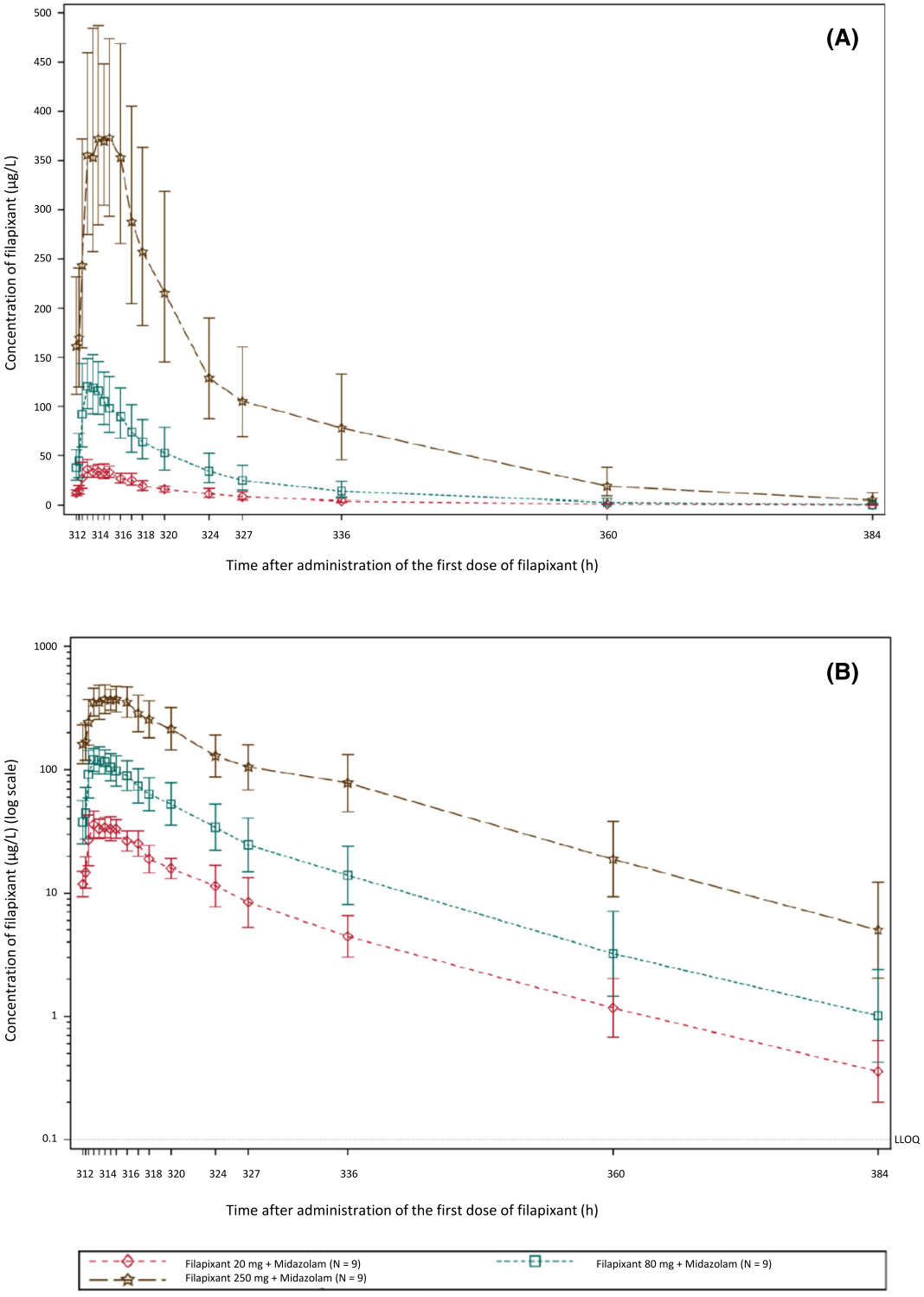

FIGURE 3 Geometric mean filapixant plasma concentration-time curves (± standard deviation) obtained in healthy young men after twice-daily administration of the drug over approximately 2 weeks: (A) linear scale and (B) semilogarithmic scale. The graph shows the filapixant concentrations after the last dose of filapixant (co-administered with a subtherapeutic dose of midazolam) (days 13-16). A graph showing the complete time course of filapixant plasma concentrations, including single-dose data and trough concentrations, is provided in Supporting Information Chapter S4, Figure S7. Abbreviations: LLOQ, lower limit of quantitation (0.1000 µg/L); N, number of participants.

administration over approximately 2 weeks (day 13) is presented in Table 5. Corresponding filapixant plasma concentration-time curves are provided in Figure 3.

The data show that filapixant was rapidly absorbed after oral administration, with median times to maximum of 1 to 2 h. The systemic exposure of filapixant increased dose-proportionally in the dose

range studied. The geometric mean dose-normalized exposure of filapixant (AUC(0-12)/D) was similar in the three dose groups after single as well as after multiple administration of the drug. The same applied to C_{max}. The results of the explorative analysis of variance also indicated a dose-proportional increase of AUC and C_{max} over the dose range studied (Supporting Information Chapter S4, Table S6). The power model provided a slope estimate of 0.9742 (90% CI 0.8886; 1.0599) for $AUC(0\text{-}12)_{md}$ and 0.9315 (90% CI 0.8676; 0.9953) for $C_{max,md}$. Hence, dose proportionality could be formally proven only for $AUC(0\text{-}12)_{md}$ by the power model. However, since the point estimate for the slope is near to 1 for C_{max} and its 90% CI only just excludes 1, dose proportionality of C_{max} may still be regarded as a valid assumption. The linearity index of filapixant, that is, the ratio of the AUC(0-12) after multiple dosing to AUC(0-∞) after single dosing, which was very close to unity, supports the assumption of time-linear kinetics.

PK steady state was reached about 72 h after first administration of filapixant in all dose groups (Supporting Information Chapter S4, Figure S7). The interindividual variability of filapixant exposure was low to moderate (geometric mean coefficient of variation 20-40% for $AUC[0\text{-}24]_{md}$ and 17-30% for $C_{max,md}$).

As expected, based on the observed half-life and the dosing interval, C_{max} and AUC(0-12) were increased after multiple dosing compared to single-dose administration. The geometric mean accumulation ratios for the 250-mg dose were 1.61 for C_{max} and 1.86 for AUC. There was also an increase in $t_{1/2}$ observed between single and multiple-dose administration, which likely reflects the shorter sampling interval after the first (single) dose, which did not allow the terminal drug-disposition phase to be fully captured.

3.4 | Safety

Treatment-emergent AEs were reported for participants from all treatment groups, including the placebo group (28 of 36 participants, 77.8%). The most frequently reported AEs (Medical Dictionary for Regulatory Activities preferred terms) were headache and, as described above, AEs related to taste perception (taste disorder without further specification and dysgeusia) (see details in Supporting Information Chapter S4, Table S7). All AEs were mild or moderate and had resolved completely by the time of the follow-up visit. No deaths or other serious AEs or AEs leading to discontinuation of treatment were reported.

In participants treated with 250 mg of filapixant, increased values of antithrombin activity were observed. Increases were up to ~30% above baseline. The effect was first observed approximately 3 days after administration of the first dose of filapixant. Bleeding or bruising was not observed apart from one case of epistaxis in the placebo group. Since, to our knowledge, increased antithrombin activity has not been linked to an increased bleeding risk in the medical literature, the increases observed in the 250-mg group were assessed as not clinically significant after consultation with experts.

No clinically relevant changes compared to placebo and baseline were noted in the other safety laboratory parameters, vital signs (including oxygen saturation) and electrocardiograms.

4 | DISCUSSION

4.1 | Introduction and brief summary of results

In this paper we present the results of safety, pharmacodynamic and PK investigations with the P2X3 receptor antagonist filapixant, focusing on changes in taste perception and cough-reflex sensitivity. The frequency of the changes in taste perception observed in this study was higher than originally expected. The results of the cough-challenge test, in contrast, were less conclusive than we had hoped for despite the small sample size. The PK profile of filapixant proved to be favourable and showed no relevant CYP3A4-interaction potential.

4.2 | Taste perception

Based on experience with other P2X3 antagonists, for example the archetype P2X3 antagonist gefapixant with its relatively low P2X3: PX2/3 receptor selectivity and eliapixant with its higher selectivity (~13-20-fold),[15] alterations in taste perception after administration of filapixant were deemed possible although not expected in this frequency, given the >100-fold higher selectivity of filapixant for P2X3 over P2X2/3 (unpublished data, Bayer AG).

In the present study, eight of nine participants (89%) from the 250-mg group reported taste-related AEs, which were all mild in intensity and did not lead to discontinuation of treatment. This incidence rate is similar to the rates observed in healthy study participants and in patients receiving supratherapeutic doses of gefapixant,[25,41] and it is considerably higher than the rate observed in healthy men receiving eliapixant (9%).[36] It is even higher than the rate observed in the above-mentioned phase 2a study in patients with RCC receiving ascending doses of filapixant (20, 80, 150 and 250 mg BID for 4 days each with 3-day drug-free intervals between dose steps) (57%, counting all taste-related AEs present at the 250-mg step, irrespective their time of onset).[1] One explanation for the high incidence of taste-related AEs in the present study might be that the participants—sensitized by the taste-strips tests—were more aware of changes in taste sensations and reported taste disturbances more often than in studies without such tests.

The subset of participants reporting the occurrence of taste-related AEs and the subset of participants describing taste impairments in the taste questionnaire overlapped; more precisely, the former included the latter. Both approaches clearly differentiated between placebo and high-dose treatment. Taste-related AEs were reported almost exclusively by participants from the 250-mg group and only participants from this group described taste impairments in the questionnaire. Comparing the taste impairments recorded in the questionnaire and the impairments recorded as AEs in terms of the taste modality affected proved to be problematic as the reports of taste-related AEs were mostly unspecific ("taste disturbance without other specification", "change in taste") or referred to taste sensations

not covered in the questionnaire. In contrast to the taste-related AE and questionnaire data, the overall and taste modality-specific taste-strips test scores showed only random changes in all treatment groups, including the placebo group.

The observed differences between the outcomes of the taste-strips test, the taste questionnaire and the taste-related AE reports could be a result of methodological differences between three approaches used for investigating taste alterations—a taste sensitivity and recognition task with four one-dimensional taste modalities versus a symptom check list versus free recall of AEs. However, they might also indicate that these three approaches complement each other, that is, capture different components of the taste sensory system. While taste-strips tests are suitable for detecting quantitative, modality-specific changes in taste perception (hypogeusia, hypergeusia), spontaneous reports of taste-related AEs may also be brought about by qualitative changes in taste perception (parageusia), which may be caused by altered signal transduction. For example, instead of a sweet yogurt the study participant may taste a slightly salty yogurt and describe this either as a different taste (eg, salty) or as less tasty than usual. A similar phenomenon has been described for chemotherapy patients.[42] Interestingly, mainly or only participants from the 250-mg group reported taste disturbances as AEs or in the questionnaire, respectively, while changes of taste-strips test scores of ±2 and more points were observed in all groups, including the placebo group. Noteworthy also is that all participants in this study underwent taste-perception screening before enrolment. Only participants who were able to correctly identify the taste of the strips at the second-highest concentration at least were eligible for participation. This led to a high screening failure rate but also enriched the cohort for tasters,[43] allowing for detection of true treatment effects on taste sensation. An option for future studies of this kind might be to use real food or meals instead of simple taste strips for taste assessments.

As mentioned above, the high incidence of taste-related AEs after administration of filapixant was not expected given the high selectivity of filapixant, but maybe the expectation that a high selectivity for P2X3 homomers is the key to avoiding taste-related AEs is based on false assumptions. High and Finger have shown in a recently published study that in most humans the nerve fibres innervating taste buds express only P2X3 homomers.[44] This finding raises a number of new questions, but it remains to be seen whether it can be confirmed in further studies. Other factors such as receptor binding mechanisms or the PK properties of the drug may also play an essential role in inducing changes in taste perception. Of note, all of the P2X3 antagonists are allosteric[45] and thus different binding sites may be involved in differences in taste-related side effects. Faster concentration changes or higher peak-trough fluctuations, for example, might lead to more taste-related AEs or more noticeable changes in taste sensations. In this context, it should also be noted that all the above information on receptor selectively is based on in vitro data using human material. As receptor selectivity is species-dependent, animal data cannot be used to predict the selectivity of a drug in humans.

4.3 | Cough challenge

The data obtained in the ATP cough-challenge test showed the expected increase in cough frequency with increasing ATP concentration. Slight overall decreases of ATP-induced cough frequencies were observed after study drug administration. However, there were no major differences in cough responses between the different filapixant doses or placebo. In contrast, a pharmacodynamic effect could be shown with the same ATP chough-challenge test in a study with the P2X3 antagonist gefapixant in healthy volunteers, although the observed effect was less pronounced than seen later in patients with chronic cough.[19,46] Our inconclusive results, however, are line with the ATP cough-challenge results of a study of eliapixant in healthy men.[36]

One explanation for the lack of effect observed in our study might be that the cough count data are biased by the participants' attempts to meet the investigator's expectations. The participants might have coughed intentionally rather than reflexively, in particular during screening when they wanted to qualify for study participation. A slight imbalance between treatment groups regarding the predose cough counts (≥5 coughs in particular) might also have contributed to the lack of effect. That is, the window to observe a treatment effect was smaller in the 80- and 250-mg groups than in the other groups (bottom effect).

The lack of effect might also be attributable to the inhalation method used, for example no flow limiter on the nebulizer was used in this study to ensure uniform delivery of the tussive agent. Variation in ATP deposition is known to increase the variability and might have masked the effect of filapixant. Another explanation for the outcome of the challenge test might be that healthy individuals generally respond less to cough challenges than patients with respiratory conditions and overactivation of sensory nerve fibres in the upper respiratory tract.[34,35] In fact, treatment with filapixant (80, 150 and 250 mg BID, 4 days on/3 days off) led to a significant reduction in cough frequency and severity in patients with RCC in the above-mentioned phase 2a study.[1]

An option for future studies of this kind could be to conduct ATP cough-challenge tests in patients with RCC, but the better option would probably be to start directly with the assessment of spontaneous coughing in the target population.

4.4 | Pharmacokinetics

The PK data collected in this study showed a favourable PK profile of filapixant: the variability of exposure is low (coefficient of variation < 30% for AUC) and the terminal half-life indicates that filapixant could also be suitable for once-daily intake. Furthermore, filapixant has no clinically relevant CYP3A4 interaction potential and the CYP3A4 induction seen in preclinical studies was not observed clinically.

The approach used to study the CYP3A4 interaction potential of filapixant can be regarded as a special strength of this study. Adding a

single PK profiling day for midazolam alone to the study design and co-administering a subtherapeutic dose of midazolam together with the potential perpetrator allows all data necessary to assess the CYP3A4 interaction potential of a drug candidate early during its clinical development to be obtained in a cost-efficient way without significantly increasing the burden on the participants.[47] Separate drug-drug interaction studies in healthy volunteers are no longer necessary when this approach is used.

4.5 | Limitations of the study

The limitations of the study are the short duration of treatment (with regard to long-term effects), the fixed sequence of treatments (with regard to the evaluation of the interaction potential), that the taste-strips test did not cover umami,[48] the fifth basic taste, and the relatively small sample size. As mentioned in the Introduction and Methods Sections 1 and 2, this early phase 1 study was primarily designed as a safety and tolerability study and was not powered for pharmacodynamic measurements. Thus, supplementary investigations will be necessary to obtain more conclusive results and complete our knowledge of the pharmacodynamic properties of filapixant. These future investigations should, of course, also include female participants as some of the intended target populations mainly or exclusively comprise women. Patients with RCC, for example, are typically middle-aged or older women.[49,50]

The inclusion of only healthy young men is another limitation of the study. Increased cough-reflex sensitivity in females[51] and sex- and age-related differences in gustatory functions have been reported.[29,52] Patients with RCC or other disorders associated with sensory-nerve-fibre overactivation are expected to have higher activity of primary afferent sensory fibres affected (eg, the airway) than healthy individuals, and therefore they might have a better responsiveness to P2X3 inhibition. Gustatory function might also be altered differently in patients with RCC than in healthy subjects. However, in a dedicated study by Nussbaum et al, the safety and tolerability profile of the P2X3 antagonist gefapixant was generally consistent between healthy younger adults and older adults with RCC.[41]

In regard to the PKs of filapixant, there are, to our knowledge, no indications of relevant differences between young and older adults, or between men and women.

5 | CONCLUSION

Overall, filapixant was safe and well tolerated, apart from mild, transient taste disorders. Such disorders occurred more frequently than expected based on in vitro receptor-selectivity data, suggesting that factors other than P2X3:P2X2/3 selectivity might also play an important role in this context. The ATP cough-challenge test showed no clear pharmacodynamic effect. Filapixant has no clinically relevant CYP3A4 interaction potential.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design and/or to the analysis and interpretation of the data. Christian Friedrich prepared the first draft of the manuscript. All authors commented on this version and later versions of the manuscript, and read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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