

Preclinical Evaluation of the Effects of Trazpiroben (TAK-906), a Novel, Potent Dopamine D₂/D₃ Receptor Antagonist for the Management of Gastroparesis

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Received for publication Apr 26, 2021. Accepted for publication Jul 01, 2021

ABSTRACT

Current therapies for gastroparesis metoclopramide and domperidone carry risks of extrapyramidal symptoms and life-threatening cardiac arrhythmias. Trazpiroben, a novel, potent dopamine D₂/D₃ receptor antagonist, has low brain permeation and very low affinity for human ether-à-go-go-related gene (hERG) channel inhibition, potentially improving on safety profiles of existing therapies. Trazpiroben demonstrated the following receptor affinities: high for D₂ and D₃, moderate for D₄, and minimal for D₁ and D₅. It demonstrated moderate affinity for adrenergic α 1B (α_{1B}) and 5-hydroxytryptamine (5HT) 2A receptors and low potential for off-target adverse events (AEs). Trazpiroben potently inhibited dopamine-activated D_{2L} receptor activation of cognate G-proteins in human embryonic kidney 293 cell membranes and was a neutral D_{2L} receptor antagonist. In vivo, trazpiroben dose-dependently increased prolactin release in orally dosed rat (0.1–1 mg/kg). Additionally, multiple oral doses in the rat (100 mg/kg) and dog (50 mg/kg) for 3 days produced robust plasma exposures and prolactin increases in both species. Trazpiroben inhibited retching/vomiting in the dog with apomorphine-induced emesis with a potency (0.1–1 mg/kg) like that of trazpiroben-mediated prolactin increases in rat. Oral trazpiroben (1, 10, and 30 mg/kg) did not affect rat

rotarod performance, suggesting low brain penetration. Trazpiroben concentrations were low in cerebrospinal fluid versus plasma after multiple oral doses for 4 days in rat and dog. Trazpiroben weakly inhibited the hERG channel current (concentration causing half-maximal inhibition of control-specific binding of 15.6 μ M), indicating little potential for disrupting cardiac rhythm. Overall, trazpiroben is a potent D₂/D₃ receptor antagonist designed to avoid the serious potential AEs associated with current gastroparesis therapies.

SIGNIFICANCE STATEMENT

Trazpiroben is a novel, potent dopamine D₂/D₃ selective receptor antagonist designed to avoid adverse effects associated with the current pharmacological therapies metoclopramide and domperidone. Preclinical studies have demonstrated low brain penetration and weak affinity for the hERG channel, indicating that trazpiroben is not expected to be associated with central nervous system or cardiovascular safety issues. With these pharmacological properties, trazpiroben may represent a viable new treatment option for gastroparesis because of a potentially improved safety profile relative to existing therapies.

Introduction

Gastroparesis, a chronic gastric motility disorder, is distinguished by delayed gastric emptying and gastric dysrhythmia with diminished peristaltic coordination in the absence of mechanical obstruction of the gastric outlet (Camilleri et al., 2018; Gharibans et al., 2019; Grover et al., 2019). Patients report chronic symptoms with periods of exacerbation, which commonly include early satiety, upper abdominal discomfort and bloating after a meal, postprandial fullness, and nausea and vomiting (Camilleri et al., 2018). Symptoms frequently mimic those observed with other conditions, such as functional dyspepsia, leading to issues in identifying and diagnosing the disease (Parkman et al., 2004; Camilleri et al., 2018; Tack and Camilleri, 2018). Gastroparesis may stem from a number of different etiologies, including idiopathic, diabetic, or

This study was sponsored by Altos Therapeutics LLC. Medical writing support was provided by Alexandra Kisbey-Ascott of Oxford PharmaGenesis, Oxford, UK and was funded by Takeda Pharmaceutical Company Ltd.

As a former shareholder of Altos Therapeutics LLC, Dr. Whiting will benefit from any future payments by Takeda with respect to certain clinical development and commercial milestones for trazpiroben. Dr. Whiting has received consulting fees from Takeda and is a current board member of VM Pharma LLC. As a former shareholder of Altos Therapeutics LLC, Dr. Luehr will benefit from any future payments by Takeda with respect to certain clinical development and commercial milestones for trazpiroben. As a former shareholder of Altos Therapeutics LLC, Dr. Jasper will benefit from any future payments by Takeda with respect to certain clinical development and commercial milestones for trazpiroben.

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dx.doi.org/10.1124/jpet.121.000698.

ABBREVIATIONS: AE, adverse event; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; CTZ, chemoreceptor trigger zone; EPS, extrapyramidal side effect; 5-HT, 5-hydroxytryptamine; HEK, human embryonic kidney; hERG, human ether-à-go-go-related gene; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; I_{Kr}, rapidly activating delayed rectifier cardiac potassium current; K_i, inhibition constant; LC-MS/MS, liquid chromatography–tandem mass spectrometry; nH, Hill coefficient.

postsurgical causes, which in turn frequently determine the reported pattern and severity of symptoms (Camilleri et al., 2018; Nassar and Richter, 2018). Rising disease severity is associated with significant morbidity and healthcare utilization; however, despite the burden associated with gastroparesis, substantial gaps remain in describing the underlying mechanisms leading to the disease, and the interplay between upper gastrointestinal symptoms and gastric emptying requires further investigation (Janssen et al., 2013; Grover et al., 2019).

In addition to challenges in identifying and diagnosing gastroparesis, the current therapeutic landscape remains limited and includes a patient-guided combination of conservative, medical, or surgical treatment options, such as dietary control, gastric electrical stimulation, and pharmacological therapies (Camilleri et al., 2018; Tack and Camilleri, 2018). These pharmacological therapies include dopamine receptor antagonists, which can reduce gastroparesis symptoms and are effective at establishing normal gastric myoelectric activity and resolving gastric dysrhythmias (Koch et al., 1989; Acosta and Camilleri, 2015; Gharibans et al., 2019). These benefits occur through reversing the actions of dopamine, which exerts a direct relaxant effect on the gut musculature through activation of muscular D_2 receptors in the lower stomach and esophageal sphincter, reducing gut motility (Lee and Kuo, 2010). Dopamine receptor antagonists also offer antiemetic benefits through preventing D_2 receptor activation in the chemoreceptor trigger zone (CTZ) (Lee and Kuo, 2010).

Two agents with dopamine receptor antagonist activity, metoclopramide and domperidone, are available for the symptomatic management of gastroparesis, although only limited use is permitted owing to safety issues. Metoclopramide, a 5-hydroxytryptamine (5-HT) 4 receptor agonist and D_2/D_3 receptor antagonist that penetrates the blood-brain barrier (BBB), carries the risk of potentially serious movement disorders known as extrapyramidal side effects (EPSs), in particular tardive dyskinesia as a result of central nervous system (CNS) D_2 receptor blockade. Hence, metoclopramide is labeled only for short-term use, and the drug carries a black box warning from the US Food and Drug Administration regarding treatment lasting longer than 3 months (Bateman et al., 1985; Parkman et al., 2012; Enweluzo and Aziz, 2013).

Domperidone is a D_2/D_3 receptor antagonist with limited brain penetration, meaning it does not elicit the same CNS adverse effects as metoclopramide and has been approved by the European Medicines Agency for short-term, low-dose use. Domperidone has been associated with QT interval prolongation, a delay in cardiac polarization that can lead to torsades de pointes, a potentially fatal form of ventricular arrhythmia, and sudden cardiac death (Hondeghem, 2011; Renoux et al., 2016; Hellstrom and Al-Saffar, 2018). Owing to these potential effects, domperidone has not been approved by the Food and Drug Administration and is only available in the United States via a single-patient Expanded Access Investigational New Drug Application. A likely mechanism for these cardiac effects is domperidone's inhibitory effect on the human ether-à-go-go-related gene (hERG) channel, with an IC_{50} of 57 nM (Claassen and Zunkler, 2005). As domperidone is primarily metabolized by the cytochrome P450 3A4 enzyme, the risk of cardiovascular adverse events (AEs) is increased when it is administered with inhibitors of this enzyme (Boyce et al., 2012; Chen and Hsiao, 2015). Consequently, there is an unmet

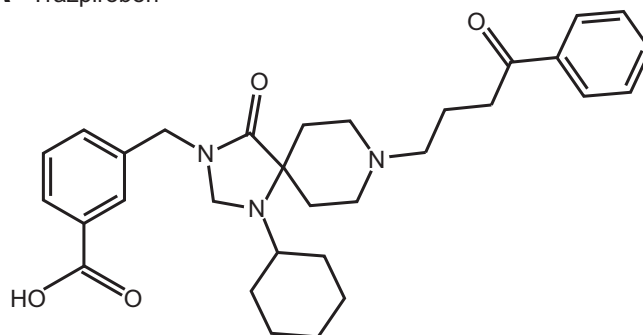
need for an effective therapy with a favorable safety profile for the management of gastroparesis.

Trazpiroben is a novel, potent D_2/D_3 receptor antagonist designed to avoid the potential AEs associated with other dopamine receptor antagonists. This article describes a series of preclinical in vitro and in vivo studies used to define the pharmacological properties of trazpiroben.

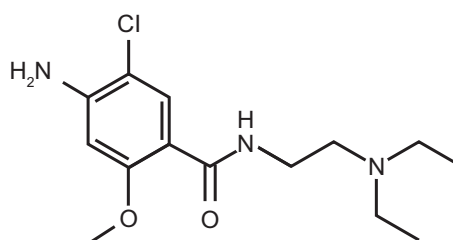
Materials and Methods

Trazpiroben, 3-[[1-cyclohexyl-4-oxo-8-(4-oxo-4-phenylbutyl)-1,3,8-triazaspiro[4.5]decan-3-yl]methyl]benzoic acid 1:1 maleic acid salt (Fig. 1), was previously known as TAK-906, ATC-1906M, and ATI-24380. ATC-1906M was synthesized at PharmaAdvance, Inc. (Jiangyin, Jiangsu, China). ATI-24380 was synthesized at ARYx Therapeutics, Inc. (Fremont, CA). ATI-24380 is the hydrochloride salt of trazpiroben. The studies and results were collected and analyzed according to a preset plan (including replicate numbers) to define the profile of the test articles in vitro and in vivo. All animal studies complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications 8023, revised

A Trazpiroben



B Metoclopramide



C Domperidone

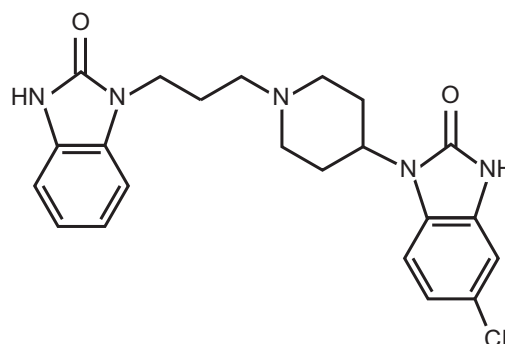


Fig. 1. Chemical structure of (A) trazpiroben, (B) metoclopramide, and (C) domperidone.

1978), the "Animal Research: Reporting of In Vivo Experiments" guidelines, and applicable animal welfare acts. Studies were approved by the local Institutional Animal Care and Use Committee. Covance Laboratories is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

For rat studies, Sprague Dawley rats from Charles River Laboratories, Raleigh, NC or from BioLasco Taiwan (under Charles River Laboratories License, Wilmington, MA) were used, whereas studies conducted in the dog made use of purebred beagle dogs from the Covance stock colony. Historical norms were used to select sample sizes for each study. Further details of the relevant animals used and their handling during the study may be found under the relevant study section.

Affinity at Human Dopamine Receptors. The affinity of trazpiroben and domperidone was examined at human D₁, D₂, D₃, D₄, and D₅ receptors using genetically engineered cell lines expressing recombinant human dopamine receptors; experiments were performed by Cerep, Inc. (Poitiers, France) according to Cerep's published protocols as described below. The affinity of trazpiroben for cloned human dopamine receptors was measured using radioligand binding displacement assays in membrane preparations from stably transfected cell lines as follows: D₁ in CHO cells (Zhou et al., 1990), D_{2S} in human embryonic kidney (HEK) 293 cells (Grandy et al., 1989), D_{2L} in human recombinant CHO cells (Grandy et al., 1989; Hayes et al., 1992), D₃ in CHO cells (MacKenzie et al., 1994), D_{4.4} in CHO cells (Van Tol et al., 1992), and D₅ in GH4 cells (Sunahara et al., 1991).

Specific ligand binding to the receptors was defined as the difference between total binding and nonspecific binding determined in the presence of an excess of unlabeled ligand. Results were expressed as the percentage of control-specific binding [(measured specific binding/control-specific binding) × 100] and as the percentage inhibition of control-specific binding (100 - [(measured specific binding/control-specific binding) × 100]) obtained in the presence of trazpiroben.

IC₅₀s and Hill coefficients (nHs) were determined using nonlinear regression analysis of competition curves generated with mean replicate values using Hill equation curve fitting [Y = D + (A - D)/(1 + (C/C₅₀)^{nH})], in which Y is specific binding, D is minimum specific binding, A is maximum specific binding, C is the compound concentration, C₅₀ is the IC₅₀, and nH is the slope factor]. This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by commercial software SigmaPlot 4.0 for Windows (1997, SPSS Inc., Chicago, IL).

Inhibition constants (K_is) were calculated using the Cheng Prusoff equation (K_i = IC₅₀/[1 + (L/K_D)], in which L is the concentration of radioligand in the assay, and K_D is the affinity of the radioligand for the receptor). A Scatchard plot was used to determine the K_D.

Receptor Specificity Profiling. Trazpiroben (as the hydrochloride salt) was tested at a single concentration of 1 μM in an affinity profiling panel of 84 receptors, and domperidone was tested at 100 nM (Cerep). Displacement of known ligands (percentage inhibition) was used to predict trazpiroben and domperidone affinity for these receptors. Binding was deemed significant if inhibition exceeded 50%. Significant binding other than that to D₂, D₃, and D₄ receptors was observed for human α_{1A}, α_{1B}, 5-HT_{1A}, 5-HT_{2A}, and 5-HT₇ receptors. Concentration-response curves were constructed with trazpiroben and domperidone at these receptors to determine affinities. Subsequently the affinities of trazpiroben (as the maleate salt) and domperidone were compared at α_{1B} and 5-HT_{2A} receptors.

The affinity at each receptor was determined at Cerep according to the following protocols: α_{1A} (Schwinn et al., 1990), α_{1B} (Ford et al., 1997), 5-HT_{1A} (Mulheron et al., 1994), 5-HT_{2A} (Bonhaus et al., 1995), and 5-HT₇ (Shen et al., 1993). For calculation of K_is, specific ligand binding to the receptors was defined as the difference between total binding and nonspecific binding measured in the presence of an excess of unlabeled ligand. Percentages of control-specific binding and inhibition of control-specific binding in the presence of trazpiroben, IC₅₀s, and nHs were determined as described in the previous section.

Functional Assay in Membrane Preparations Expressing the D_{2L} Receptor. Agonist and antagonist activities at the D_{2L} receptor were assessed using the screening protocol in the DELFIA GTP-Binding Kit (AD0167; Perkin Elmer, Waltham, MA) using Eu-labeled GTP reagents AD0260 and buffers AD0261. Membranes were prepared from HEK293 cells stably transfected with human D_{2L} receptors, and protein concentration was determined using the DC Protein Assay (500-0116; Bio-Rad, Hercules, CA). The membrane preparation was used at 10 μg per well in AcroWell 96-well filter plates (Pall Life Sciences, Port Washington, NY). Dopamine (positive control) and test compounds [trazpiroben (as the hydrochloride salt), domperidone, and metoclopramide] were evaluated at 0.3 nM–30 μM and 0.01 nM–1 μM (in serum-free medium), respectively, in a volume of 20 μl. The reagents were reconstituted and added according to the kit insert. Eu fluorescence was measured on an EnVision reader (Perkin Elmer) at 615 nm using a 340-nm excitation wavelength after 6 hours (or after the plate had completely dried).

For the antagonist protocol, test compounds were incubated for 15 minutes with the membranes, and 1 μM dopamine was used. For each concentration of antagonist tested, the IC₅₀ was determined using the Sigmoidal concentration-response (variable slope) equation (Prism, GraphPad Software, San Diego, CA).

Prolactin Levels in Rat after a Single Dose of Trazpiroben or Domperidone. Single doses of trazpiroben or domperidone were administered to male Sprague Dawley rats via oral gavage at doses of 0 (vehicle control), 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg. The rats were from Charles River Laboratories, Raleigh, NC; weighed 205–249 g; and were 7–8 weeks of age at time of study. Certified Rodent Diet 2016C (Harlan) and fresh water were provided ad libitum. Serum prolactin concentrations, as a biomarker for peripheral D₂ receptor antagonism, were measured in blood samples collected at 1 hour postdose, and levels were compared in animals receiving the two compounds. These studies were performed at Covance Laboratories, Madison, WI.

Concentrations of Prolactin in Serum after Multiple Oral Doses of Trazpiroben to Rat. In the rat, after a 4-day acclimatization period, individual doses of trazpiroben were calculated based on body weights measured on the first day of dosing. The study involved five male and five female Sprague Dawley rats. Rats were dosed by oral gavage once daily at 100 mg/kg. Blood was collected for serum prolactin determination as follows: day 1, predose and at 1, 6, and 24 hours postdose, and day 3, predose and at 1, 6, and 24 hours postdose. Daily observations of the health of the animals were made.

Trazpiroben was prepared in 5% (v/v) dimethyl sulfoxide, 40% (v/v) polyethylene glycol 400, and 55% (v/v) 0.9% sodium chloride for injection on study day 1 for all doses. When not in use the formulations were stored at 2–8°C, protected from light. Rats were dosed at 100 mg/kg via a ball-tipped gavage needle for 4 consecutive days. For determination of prolactin concentration, blood (approximately 0.4 ml) was collected from a jugular vein via a syringe and needle and transferred into serum separator tubes on days 1 and 3 predose and at 1, 6, and 24 h (prior to the next daily dose, as applicable) postdose. Blood samples intended for prolactin quantification were processed to serum, frozen, and stored at -70°C (±15°C) until analysis.

All samples were evaluated for prolactin using an assay based on the competition between unlabeled prolactin and a fixed quantity of 125I-labeled prolactin for a limited number of binding sites on the prolactin-specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of ligand bound by the antibody was inversely proportional to the concentration of prolactin in the sample. The antibody-bound prolactin was then reacted with a second antibody bound to a magnetizable particle. Separation of the antibody-bound fraction was accomplished via centrifugation and decantation of the supernatant. Measurement of the radioactivity in the pellet allowed the amount of labeled prolactin in the bound fraction to be calculated (lower limit of quantification for prolactin was 0.8 ng/ml). Concentration of the unlabeled prolactin was then determined by interpolation from the standard curve, and results were entered into the ClinAxy

system v2.6.1. (Clinical Systems Ltd., FL). Assays were performed at Antech Diagnostics (Morristown, NJ).

Concentrations of Prolactin in Serum after Multiple Oral Doses of Trazpiroben to Dog. This study included five male and five female purebred beagles (non-naïve) from the Covance stock colony. Animals were individually housed in steel cages during treatment and at least 4 hours after each dose administration to allow monitoring of any test article-related effects. Certified Canine Diet 5007 (PMI) was provided ad libitum. Diets were supplemented with canine treats, and water was provided fresh daily ad libitum. Following a 3-day acclimatization period, individual doses of trazpiroben were calculated based on body weights measured on the first day of dosing (8.3–12.5 kg). Trazpiroben was prepared in capsules (size 12, one-fourth ounce) by Covance on study day 1 for all doses. When not in use, the formulations were stored at ambient temperature and protected from light. Dogs were then dosed via oral capsule (five capsules followed by at least 10 ml of water) once daily at 50 mg/kg for 4 consecutive days. Blood (approximately 1 ml) was collected from a jugular vein for serum prolactin determination as follows: day 1, predose and at 1, 6, and 24 hours postdose, and day 3, predose and at 1, 6, and 24 hours postdose. Daily observations of the health of the animals were made. Handling of blood samples was as described for the rat.

All samples were evaluated for prolactin using a solid phase enzyme immunoassay in the microplate format designed for the quantitative measurement of canine prolactin (lower limit of quantification for prolactin was 0.2 ng/ml). The standard curve demonstrated a direct relationship between optical density and prolactin concentration, which allowed for determination of prolactin concentration in controls and unknowns using the SoftMax Pro software (Molecular Devices, Sunnyvale, CA). Results were entered into the ClinAxys system v2.6.1 for reporting. Assays were performed at Antech Diagnostics (Morristown, NJ).

Apomorphine-Induced Emesis in Dog. Groups of male and female beagle dogs (two male and two female per group) weighing 8–15 kg were used. Animals were fasted overnight before the experiment (water only). The dogs were administered 0.5 ml/kg of trazpiroben oral solution (0.03, 0.1, 0.3, or 1 mg/kg as the hydrochloride salt) or vehicle (10% dimethyl sulfoxide, 40% hydroxypropyl- β -cyclodextrin in sterile water), which was followed 1 hour later by a single subcutaneous injection of 0.3 ml/kg apomorphine hydrochloride (0.3 mg/kg), a highly emetogenic compound. Domperidone and metoclopramide (oral solution) were used as comparators. The latency to first retch and vomit and the number and time of vomit episodes were recorded for 60 minutes after apomorphine injection. Vomiting was defined as oral expulsion of liquid or solid stomach contents. Meaningful differences were evaluated using nonparametric methods (Wilcoxon rank sum test).

Accelerating Rotarod Performance in Rat. Male Sprague Dawley rats weighing 180 ± 30 g were obtained from BioLasco Taiwan (under Charles River Laboratories License, Wilmington, MA). Trazpiroben was dissolved in 5% dimethyl sulfoxide/40% polyethylene glycol 400/55% saline. The dosing volume was 10 ml/kg. Groups of eight rats were included in the study based on predose rotarod performance. Animals were trained on a turning rod rotating at a continuous accelerating speed from 4 to 36 rpm over 4 minutes for at least three times on day 0. Rats were randomly assigned to different groups with similar baseline training values. The vehicle; trazpiroben at 1, 10, and 30 mg/kg; and chlorpromazine at 30 mg/kg were orally administered on day 1. Then, 30 and 60 minutes later, the animals were placed on the accelerating rotarod (increasing from 4 to 36 rpm during the 4-minute period). The time (in seconds) the animal remained on the turning rotarod was recorded. One-way analysis of variance followed by Dunnett's test was applied for comparison between the vehicle control group and test compound-treated groups. $P < 0.05$ was considered statistically meaningful.

Concentrations of Trazpiroben in Plasma after Single and Multiple Oral Doses to Rat and Dog. These two studies examined the effect of initial and multiple oral administration of once-daily

trazpiroben 100 and 50 mg/kg in the rat and dog, respectively, on plasma trazpiroben levels to determine systemic exposure. These studies were performed at Covance Laboratories as part of the same experiments described earlier for prolactin concentrations, and details of animals, dosing, and the timing of blood collection were as summarized previously.

In both rat and dog experiments, blood (0.25 ml rat, 1.0 ml dog) for determination of trazpiroben concentration was collected from a jugular vein via a syringe and needle and transferred into tubes containing potassium (K_2) EDTA anticoagulant on days 1 and 3 predose and at 1, 6, and 24 (prior to the next daily dose, as applicable) hours postdose. On day 4, blood (0.25 ml rat, 1.0 ml dog) was collected via cardiac puncture under isoflurane anesthesia for rat and from a jugular vein for dog at 1 hour postdose, with cerebrospinal fluid (CSF) also collected at 1 hour postdose and 1 and 3 hours postdose for the rat and dog, respectively, into tubes containing potassium (K_2) EDTA anticoagulant.

Blood samples intended for determination of trazpiroben concentration using (K_2) EDTA anticoagulant were processed to plasma via centrifugation, stored at -70°C , and shipped frozen to the bioanalytical laboratory (Covance NWT, West Trenton, NJ). Analyst software (version 1.6.2, Sciex, Framingham, MA) was used for acquisition of liquid chromatography–tandem mass spectrometry (LC-MS/MS) data and integration of trazpiroben and warfarin internal standard chromatographic peaks. The Watson LIMS (version 7.4.1, Thermo-Fisher Scientific, Waltham, MA) laboratory information management system was used for data storage, management, study design, sample receipt, interfacing with instruments, regression of analytical results, and reporting of results.

For analysis, blood was first processed to plasma via protein precipitation, and then 50 μl plasma and 50 μl of warfarin internal standard solution (100 ng/ml) were covered with aluminum foil and vortex mixed for at least 30 seconds. Next, 500 μl of 1% formic acid in acetonitrile was added to the sample, which was then vortexed for approximately 3 minutes and followed by centrifugation at approximately 1600 g for 5 minutes at room temperature. A 50- μl aliquot of the supernatant was transferred to a 96-well plate that contained 350 μl methanol:water (50:50, v:v). The plate was covered with a dimpled sealing mat and vortex-mixed at low speed for approximately 1 minute.

An injection volume of at least 5 μl but not more than 25 μl was injected into the LC-MS/MS. The method had a quantification range of 1.00 to 1000 ng/ml. Calibration standard concentrations were 1, 2, 7.5, 25, 100, 400, 850, and 1000 ng/ml. Quality-control sample concentrations were 1, 3, 50, 750, and 5000 ng/ml. The maximum dilution factor was 10.

The high-performance liquid chromatography column was a Waters xBridge C18 50 \times 2.1 mm column (Milford, MA) with 5- μm particle size maintained at 35°C . The mobile phase was as follows: A, 5 mM ammonium formate in 0.1% formic acid in water, and B, 0.1% formic acid in methanol. The initial flow was 0.600 ml/min at 50% B and was increased to 95% B and then back to 50% B over a cycle time of approximately 4.5 minutes (injection start to next injection start).

Mass spectrometer was a triple quadrupole Sciex API 5000/5500 using positive ion electrospray ionization in multiple reaction monitoring mode. The monitored trazpiroben mass transition was 518.4 to 244.3, and the warfarin internal standard transition was 309.1 to 163.1. The calibration curve was obtained by linear regression on trazpiroben/warfarin peak area ratio with a weighting factor of $1/x^2$. Trazpiroben sample concentrations were reported in ng/ml.

The method criteria for calibrator precision and accuracy was $\geq 75\%$ of standards within $\pm 15\%$ ($\pm 20\%$ lower limit of quantification), and the criteria for quality-control precision and accuracy was $\geq 75\%$ of standards within $\pm 15\%$. Matrix freeze-thaw stability was four cycles, sample collection stability was 2 hours at room temperature and on wet ice, and frozen matrix stability was at least 114 days at -10 to -30°C and at least 114 days at -60 to -80°C .

Plasma Protein Binding. Plasma protein binding of trazpiroben was determined in rat (Sprague Dawley) and dog (beagle) plasma using equilibrium dialysis with high-performance LC-MS/MS quantification. The concentration of the test compound was 10 μ M. A reference panel of other compounds (acebutolol, quinidine, and warfarin) with known plasma protein binding was also run at the same concentration.

Concentrations of Trazpiroben in Cerebrospinal Fluid after Multiple Oral Doses to Rat and Dog. These studies examined the effect of multiple oral administration of once-daily trazpiroben 100 mg/kg in rat and 50 mg/kg in dog on CSF trazpiroben levels to estimate brain exposure. These studies were performed at Covance Laboratories as part of the same experiments described earlier for plasma determinations of trazpiroben levels, and details of animals, dosing, and the timing of blood collection were as summarized there.

For determination of trazpiroben CSF concentrations, on day 4, for rat as much CSF as possible was collected from the cisterna magna of each animal during terminal procedures via a syringe at 1 hour post-dose. For dog, the animals were anesthetized with propofol (intravenous, slow bolus), and CSF (approximately 0.5 ml) was collected from the cisterna magna of each dog via a syringe and needle and transferred into 96-well tubes at 1 and 3 hours postdose. High- and low-range LC-MS/MS was used for quantification of trazpiroben as described previously for plasma, with samples prepared in the same way.

Effects of Trazpiroben on the hERG Channel Current. In vitro effects of trazpiroben were assessed on the hERG channel current [a surrogate for the rapidly activating delayed rectifier cardiac potassium current (I_{Kr}); (Redfern et al., 2003)]. The study complied with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Guidance for Industry (July 2001), "S7A Safety Pharmacology Studies for Human Pharmaceuticals" and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Guidance for Industry (October 2005), and "S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals." The study was conducted in accordance with published procedures (Kirsch et al., 2004) and with the standard operating procedures of ChanTest Corporation (Cleveland, OH).

The concentration-response relationship for the effect of trazpiroben on the hERG potassium channel current was evaluated at near-physiologic temperature in stably transfected mammalian cells expressing the hERG gene. In this assay, hERG potassium channels were stably expressed in a HEK293 cell line lacking endogenous I_{Kr} . Cells were transferred to the recording chamber and superfused with vehicle control solution. Micropipette solution for whole-cell patch clamp recordings comprised (mM) potassium aspartate, 130; MgCl₂, 5; EGTA, 5; ATP, 4; and HEPES, 10, with pH adjusted to 7.2 with KOH. Recording took place at 33–35°C using a combination of inline solution pre-heater, chamber heater, and feedback temperature controller. Cells stably expressing hERG were held at –80 mV. Onset and steady-state inhibition of the hERG potassium current due to trazpiroben were measured using a pulse pattern with fixed amplitudes [conditioning prepulse +20 mV for 1 second; repolarizing test ramp to –80 mV (–0.5 V/s) repeated at 5-second intervals]. Each recording ended with a final application of a supramaximal concentration of reference substance to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted offline digitally from the data to determine the potency of the test substance for hERG inhibition.

Trazpiroben was applied to each cell ($n = 3$) at different concentrations. Peak current was measured during the test ramp and a steady state was maintained for at least 20 seconds before applying test compound or positive control. Peak current was measured until a new steady state was achieved. Test compound concentrations verified by dose solution analysis were used in IC₅₀ calculations.

Domperidone was also tested by ChanTest Corporation in duplicate using the automated FASTPatch hERG concentration-response assay

in HEK293 cells expressing the human hERG channel using an automatic parallel patch clamp system (PatchXpress 7000A; Molecular Devices, San Jose, CA) according to the standard operating procedures for this method.

Results

Affinity at Dopamine Receptors. Trazpiroben exhibited high affinity for D₂ and D₃ receptors ($K_i = 1.5$ nM for D_{2S}, 3.1 nM for D_{2L}, and 3.2 nM for D₃), moderate affinity for D₄ receptors ($K_i = 80$ nM for D_{4.4}), and very weak affinity for D₁ and D₅ receptors (negligible affinity observed at 1 μ M) in stably transfected cell membrane preparations. The affinity of trazpiroben for the human D_{2S} and D₃ receptors ($K_i = 1.5$ nM and 3.2 nM, respectively) was similar to that of domperidone (0.70 nM and 2.3 nM, respectively; Fig. 2).

Receptor Specificity Profiling. Trazpiroben had affinity for α -adrenergic (α_{1A} and α_{1B}) and serotonergic (5-HT_{1A}, 5-HT_{2A}, and 5-HT₇) receptors at the screening concentration of 1 μ M. It had negligible affinity for the additional molecular targets tested, suggesting a low probability of off-target AEs.

Concentration-response curves were constructed with trazpiroben at these receptors to determine affinities. Trazpiroben affinity for each of these receptors was low for α_{1A} ($K_i = 150$ nM), 5-HT_{1A} ($K_i = 230$ nM), and 5-HT₇ ($K_i = 1.5$ μ M) receptors and moderate for α_{1B} ($K_i = 30$ nM) and 5-HT_{2A} ($K_i = 66$ nM) receptors. Domperidone had a similar receptor affinity profile to trazpiroben, with low affinity at α_{1A} ($K_i = 110$ nM) and 5-HT₇ (62% inhibition at 0.1 μ M) receptors and moderate affinity at α_{1B} ($K_i = 16$ nM) and 5-HT_{2A} ($K_i = 19$ nM) receptors. These results demonstrated a receptor binding profile for trazpiroben that closely resembled that of domperidone, although these compounds have distinctly different molecular structures.

Functional Assay in Membrane Preparations Expressing the D_{2L} Receptor. Functional activity of trazpiroben at the D_{2L} receptor was determined as G-protein activation in comparison with the agonist dopamine by measurement of Eu-GTP binding in fluorescence assays (Fig. 3). Trazpiroben did not act as an agonist or partial agonist at D_{2L} receptors in this assay (Fig. 3A). Trazpiroben and domperidone are D_{2L} receptor antagonists with IC_{50s} of 1.8 nM and 1.7 nM, respectively (Fig. 3B). Thus, trazpiroben is a neutral antagonist at the D_{2L} receptor.

Prolactin Levels in Rat after a Single Dose of Trazpiroben or Domperidone. Trazpiroben produced increases in serum prolactin concentrations in rat relative to concurrent control values after oral gavage doses of 0.1–10 mg/kg. A maximal increase in serum prolactin concentrations was observed in animals administered 1 mg/kg, and increases of similar magnitude were seen at 3 and 10 mg/kg (Fig. 4).

Domperidone produced increases in serum prolactin concentrations in rat relative to concurrent control values after oral gavage doses of 1–10 mg/kg. Increases in serum prolactin levels observed in rat administered 1, 3, and 10 mg/kg were similar in magnitude and were also similar to those observed after trazpiroben dosing (Fig. 5).

Concentrations of Prolactin in Serum after Multiple Oral Doses of Trazpiroben to Rat and Dog. Oral administration of trazpiroben increased serum prolactin concentrations in both male and female rats (Fig. 5A) and male and female dogs (Fig. 5B) relative to predose concentrations

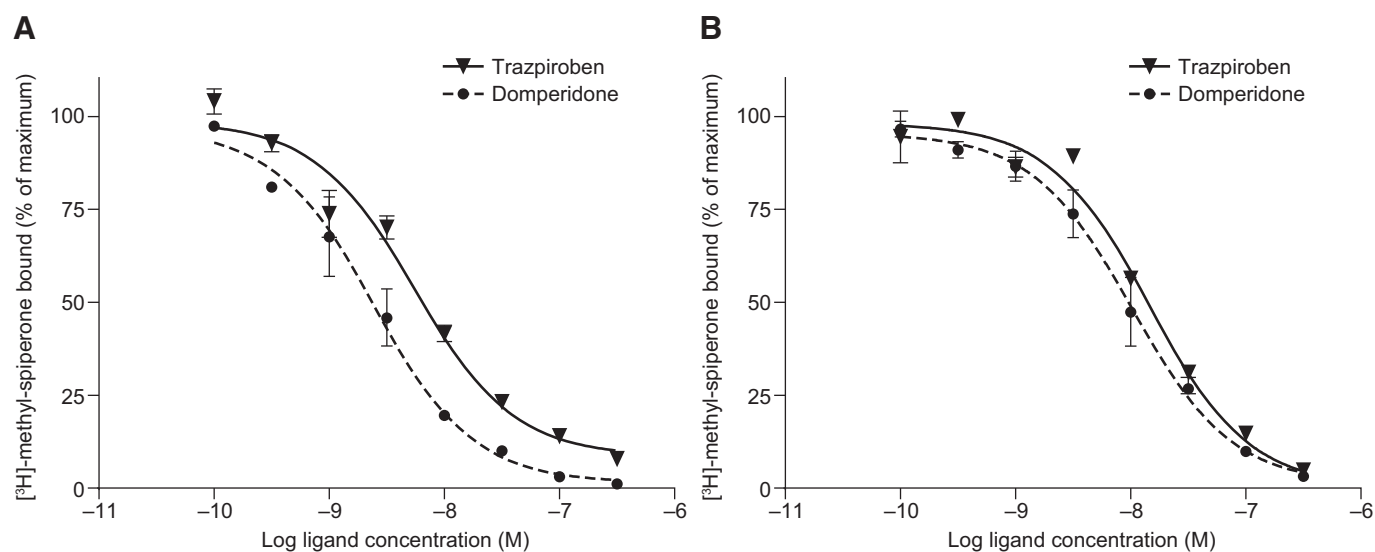


Fig. 2. Effect of trazpiroben and domperidone on human D_{2S} and D_3 receptor binding (representative graphs). Data represent the mean (\pm S.D.) concentration-dependent displacement of (A) $[^3H]$ -methyl-spiperone by trazpiroben and domperidone for the D_{2S} receptor and (B) $[^3H]$ -methyl-spiperone by trazpiroben and domperidone for the D_3 receptor.

from the initial dose onwards. On day 1, mean serum prolactin concentrations increased relative to predose concentrations and remained elevated at all subsequent time points. Maximal increases in mean serum prolactin concentrations were observed at 1 hour postdose on days 1 and 3. Mean serum prolactin concentrations at 1 hour postdose on day 1 were approximately 10- and 20-fold greater than predose concentrations in male and female rats, respectively, and 3- and 6-fold greater in male and female dogs, respectively. In both rat and dog, prolactin levels postdose on day 3 were similar to those on day 1.

Apomorphine-Induced Emesis in Dog. Apomorphine-induced emesis in beagle dogs is a well established preclinical model for testing the emetic potential of compounds (Parkinson and Grasso, 1993). Emetic response in the dog and

humans is, among other factors, under the control of D_2 receptors in the CTZ, which is located outside the BBB. In this parallel, single-dose administration study, trazpiroben resulted in statistically significant decreases in retching and vomiting compared with vehicle-treated dog [$P = 0.0078$; differences evaluated using nonparametric Wilcoxon rank sum test (Fig. 6)] at 0.3- and 1-mg/kg doses and was at least equipotent in antiemetic activity to domperidone.

Accelerating Rotarod Performance in Rat. Trazpiroben dosed at 1, 10, and 30 mg/kg orally did not affect motor coordination 30 and 60 minutes after its administration in the rat. In comparison, the centrally acting D_2 receptor antagonist chlorpromazine given orally at 30 mg/kg substantially reduced the time rats spent on the accelerating rotarod 30 and 60 minutes postdose (Fig. 7).

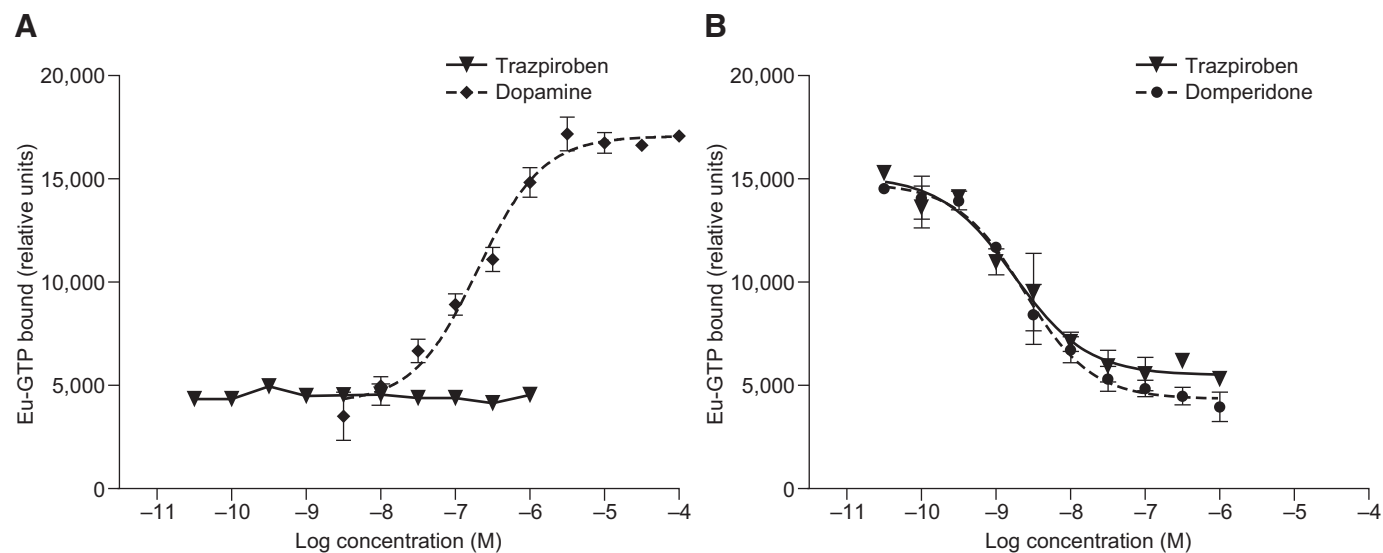


Fig. 3. Functional activity of trazpiroben in HEK293 cells expressing the human D_{2L} receptor. Data represent mean (\pm S.D.) (A) agonist (representative curves) and (B) antagonist activity of trazpiroben, dopamine, and domperidone in a time-resolved fluorometric assay.

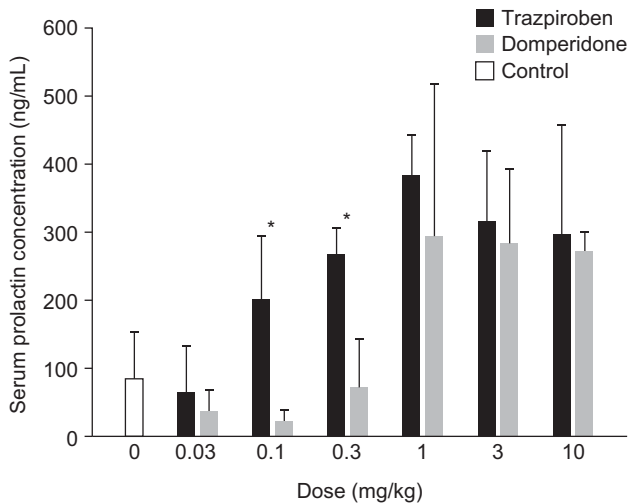


Fig. 4. Effects of trazpiroben and domperidone on prolactin levels in male rats. Data represent the mean (\pm S.D.) of serum prolactin levels 1 hour postdose after single-dose oral administration of vehicle, trazpiroben, or domperidone [$n = 4$ (rats per group)]. T-tests between the compounds indicated significant differences for prolactin levels between trazpiroben and domperidone at two doses: 0.1 mg/kg ($p = 0.0084$) and 0.3 mg/kg ($p = 0.0026$). * $P < 0.05$ was considered statistically meaningful.

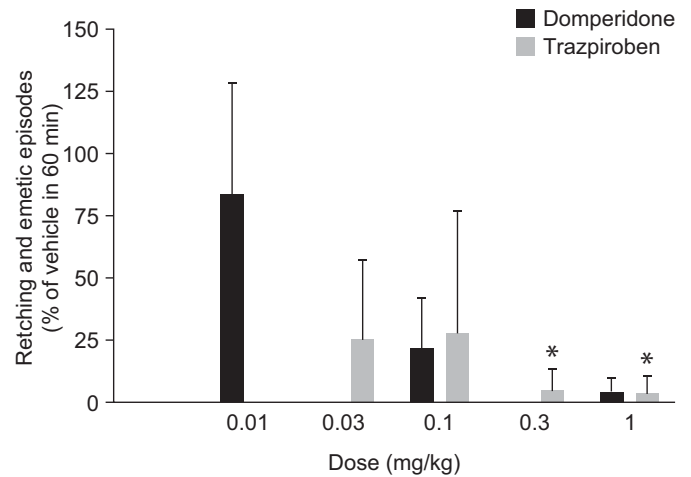


Fig. 6. Antiemetic activity of oral trazpiroben against apomorphine-induced vomiting in the beagle dog. Groups of dogs ($n = 3-4$) were treated orally with trazpiroben (0.03, 0.1, 0.3, and 1 mg/kg) or domperidone (0.01, 0.1, and 1 mg/kg). Data represent the mean vomiting and retching episode number (in % of vehicle in 60 minutes) \pm S.D. Meaningful differences were evaluated using nonparametric methods (Wilcoxon signed rank sum test). * $P = 0.0078$ at 0.3 and 1 mg/kg for dogs receiving trazpiroben versus vehicle.

Concentrations of Trazpiroben in Plasma after Single and Multiple Oral Doses to Rat and Dog. Trazpiroben was rapidly absorbed after the initial dose and each repeat dose in rat and dog. High plasma concentrations of trazpiroben were observed in all animals at 1 and 6 hours after administration on days 1 and 3 (Table 1). At 24 hours after dosing, plasma concentrations of trazpiroben were close to the lower limit of quantification (measured on days 1 and 3). Maximal mean plasma trazpiroben concentrations were higher in female than in

male rats. Concentrations in each animal were more variable in dog than in rat, and it was not possible to determine differences between the sexes. Concentrations of trazpiroben after multiple doses did not differ greatly from that measured after the initial dose in both rat and dog (Table 1).

Plasma Protein Binding. The distribution of trazpiroben was derived from in vitro studies of plasma protein binding. Plasma protein binding was 93% in rat plasma and 92% in dog plasma.

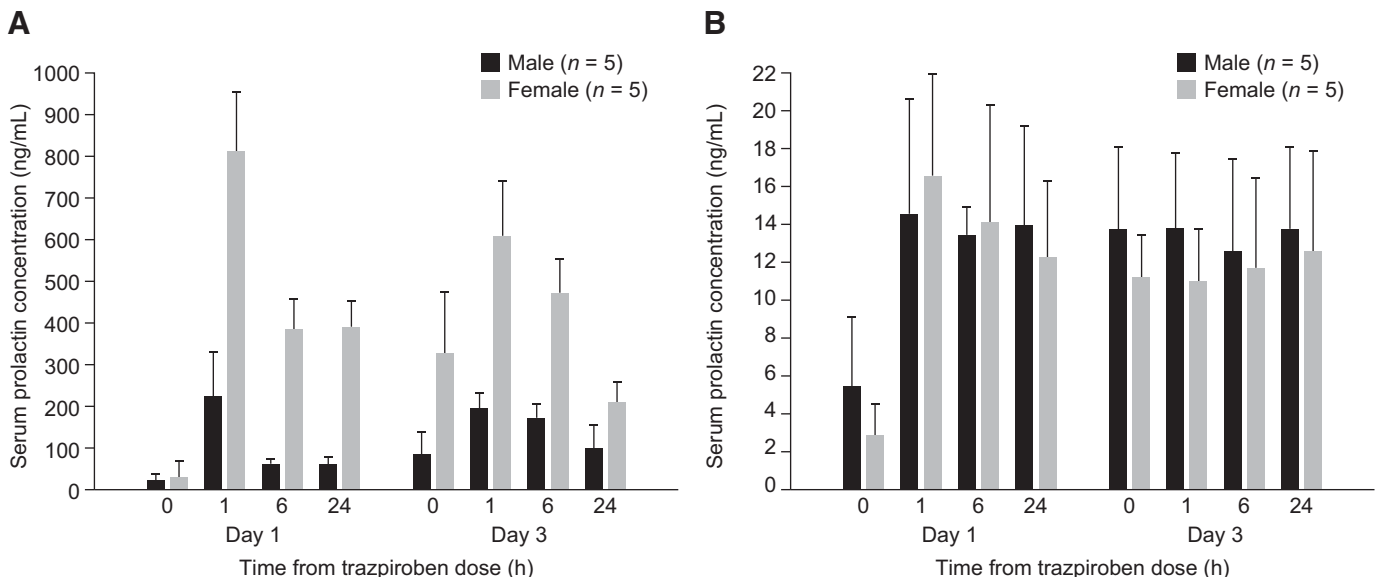


Fig. 5. Concentrations of prolactin in serum after multiple oral doses of trazpiroben to the rat and dog ($n = 5$ [rats or dogs per group, respectively]). Oral administration of trazpiroben increased serum prolactin concentrations in both male and female rats (A) and male and female dogs (B) relative to pre-dose concentrations. TAK-906, a dopamine D₂/D₃ receptor antagonist with minimal brain penetration for gastrointestinal disorders. *FASEB J.* 34, 1. (Jasper and Whiting, 2020).

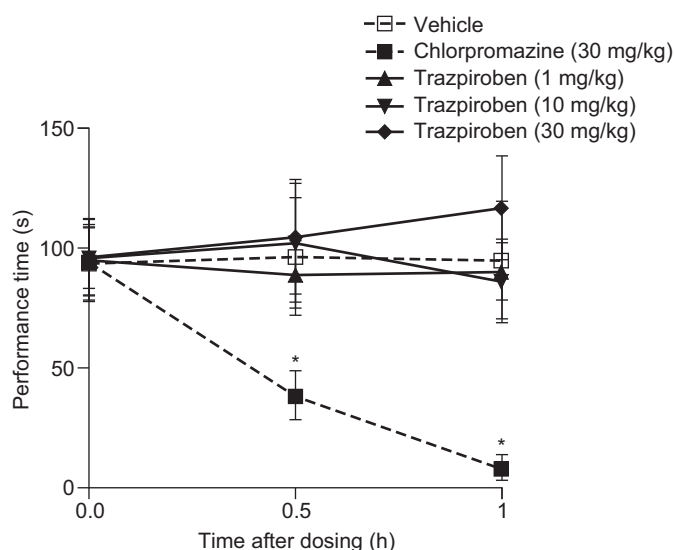


Fig. 7. Effect of trazpiroben on accelerating rotarod performance in the rat [$n = 8$ (rats per group)]. Trazpiroben dosed orally at 1, 10, and 30 mg/kg did not affect motor coordination 30 and 60 minutes after administration. In comparison, the centrally acting D_2 receptor antagonist chlorpromazine dosed orally at 30 mg/kg substantially reduced the time on the accelerating rotarod 30 and 60 minutes postdose. Data represent the mean performance time (\pm S.D.) at each timepoint postdose. One-way analysis of variance followed by Dunnett's test was applied for comparison between the vehicle control group and test compound-treated groups. * $P < 0.05$ was considered statistically meaningful.

Concentrations of Trazpiroben in CSF after Multiple Oral Doses to Rat and Dog. CSF concentrations of trazpiroben were very low compared with plasma concentrations (Table 2), indicating minimal CNS penetration and differentiating trazpiroben from metoclopramide. The ratio of plasma to CSF concentration was approximately 700:1 at 1 hour postdose on day 4 in both rat and dog and approximately 250:1 at 3 hours postdose on day 4 in the dog.

Effects of Trazpiroben on the hERG Channel Current. In good laboratory practice manual patch clamp electrophysiology studies of mammalian cells, trazpiroben only weakly blocked the hERG potassium channel current (Table 3; when fitted to a curve, the IC_{50} was 15.6 μ M with an nH of

1.3, curve not shown) relative to the positive control compound terfenadine (mean \pm S.D., $82.7 \pm 0.3\%$ inhibition at 60 nM), representing a 10,000-fold selectivity compared with the affinity (1.5 nM) at the target D_2 receptor. In a separate study, domperidone inhibited the hERG current more than 100-fold more potently than trazpiroben, with an IC_{50} of 120 nM (unpublished data). This potency for hERG current inhibition by domperidone is similar to the previously reported value of 57 nM (Claassen and Zunkler, 2005).

Discussion

Gastroparesis is a gastric motility disorder typified by gastric dysrhythmia and slow gastric emptying without mechanical obstruction (Koch, 2014; Camilleri et al., 2018; Gharibans et al., 2019). Despite the associated disease burden and healthcare resource utilization, pharmacological treatments remain limited, and those available carry safety concerns. Trazpiroben, a selective D_2/D_3 receptor antagonist developed for the chronic treatment of gastroparesis, was designed to avoid the safety issues present with current therapies. In these preclinical studies, the pharmacological properties of trazpiroben were evaluated to assess its viability as a treatment of gastroparesis.

Dopamine receptor antagonists offer effective treatment of gastric motility disorders owing to the role of D_2 and D_3 receptors in the upper gastrointestinal tract and area postrema (Darmani et al., 1999; Kashyap et al., 2009; Lee and Kuo, 2010). D_2 receptor antagonists disinhibit acetylcholine release and increase lower esophageal sphincter and gastric tone, promoting gastric emptying. D_3 receptors also play a role in gastric motility, with D_2/D_3 receptor activation significantly delaying gastric emptying in the rat, which was partially reversed by administration of a D_3 antagonist (Kashyap et al., 2009). In the area postrema, D_2/D_3 receptor antagonism has been shown to reduce nausea and vomiting (Yoshikawa et al., 1996; Darmani et al., 1999; Welliver, 2014). Importantly, as the area postrema lies outside the BBB, peripherally selective D_2/D_3 antagonists can still provide antiemetic benefits (Andrews et al., 1990; Yoshikawa et al., 1996). As a peripherally selective D_2/D_3 antagonist, trazpiroben is expected to normalize gastric myoelectric rhythm, exhibit prokinetic effects,

TABLE 1

Concentrations of trazpiroben (ng/ml) in plasma on days 1 and 3 after multiple oral doses in the rat and dog

Day	Sex of Animal	Mean (S.D.) Plasma Trazpiroben Concentration (ng/ml)			
		Predose	1 h postdose	6 h postdose	24 h postdose
Rat (100 mg/kg/day)					
1	Male	N/A	1026 (672)	1343 (686)	4.61 (3.42)
	Female	N/A	1748 (768)	2195 (1613)	14.40 (7.60)
	Male + female ^a	N/A	1387 (779)	1769 (1252)	9.49 (7.56)
3	Male	4.13 (1.92)	735 (160)	826 (302)	2.80 (1.79)
	Female	12.2 (6.10)	1225 (991)	2121 (1360)	6.26 (2.79)
	Male + female ^a	8.19 (6.04)	980 (717)	1474 (1153)	4.53 (2.86)
Dog (50 mg/kg/day)					
1	Male	N/A	1213 (1559)	182 (203)	6.70 (9.43)
	Female	N/A	2379 (4290)	196 (217)	3.62 (2.37)
	Male + female ^a	N/A	1796 (3105)	189 (198)	5.16 (6.68)
3	Male	10.5 (16.7)	3829 (2648)	333 (307)	19.60 (37.20)
	Female	74.4 (125)	2853 (1962)	181 (205)	4.09 (5.38)
	Male + female ^a	42.5 (90.7)	3341 (2256)	257 (259)	11.80 (26.30)

N/A, not applicable.

^aCombined mean (S.D.) for males and females. Five male and five female animals were used in each experiment.

TABLE 2
Concentrations of trazpiroben in plasma and CSF after multiple oral doses in the rat and dog on day 4

Animal	Trazpiroben Concentration (ng/ml), Mean (S.D.)			
	Plasma		CSF	
	1 h postdose	3 h postdose	1 h postdose	3 h postdose
Rat (trazpiroben 100 mg/kg/day)				
Male	916 (413)	–	0.99 (1.11)	–
Female	1475 (615)	–	2.42 (1.03)	–
Male + female ^a	1195 (575)	–	1.70 (1.26)	–
Dog (trazpiroben 50 mg/kg/day)				
Male	2698 (3115)	2145 (973)	2.94 (3.00)	8.93 (4.07)
Female	1991 (2514)	1832 (1771)	3.71 (3.67)	6.19 (3.74)
Male + female ^a	2345 (2695)	1989 (1357)	3.33 (3.19)	7.56 (3.96)

–, not available.

^aCombined mean (S.D.) for males and females.

and reduce nausea and vomiting without the potential CNS effects observed with other therapies. This multimodal action renders trazpiroben a promising therapeutic agent for treating gastroparesis.

Adverse effects associated with other D₂/D₃ antagonists have limited their use when treating gastroparesis in the clinic (Meltzer, 2013; Michaud and Turgeon, 2013; Giudicessi et al., 2018). Metoclopramide is a D₂/D₃ antagonist and 5-HT₄ agonist approved and marketed in the United States for treatment of symptomatic gastroesophageal reflux and diabetic gastroparesis (maximum 12 weeks' duration) (US Food and Drug Administration). Despite its efficacy, the dose and duration of metoclopramide treatment are limited by well documented toxicities, most notably EPS (Meltzer, 2013). Trazpiroben does not readily penetrate the BBB and is therefore unlikely to elicit such adverse events. Domperidone, an antiemetic and prokinetic agent, is a D₂/D₃ antagonist that inhibits the hERG channel and is associated with a risk of drug-induced long QT syndrome, torsades de pointes, and sudden cardiac death (Straus et al., 2005; Michaud and Turgeon, 2013; Giudicessi et al., 2018). Relative to domperidone, trazpiroben very weakly inhibits the hERG channel, reducing the potential for fatal arrhythmias. Owing to its low BBB penetration and weak hERG affinity, trazpiroben is anticipated to have an improved safety profile over metoclopramide and domperidone while preserving the high-affinity D₂/D₃ receptor antagonism underpinning the effectiveness of these therapies.

Numerous *in vitro* assays were employed to characterize trazpiroben. Radioligand binding assays demonstrated its high affinity (single-digit nanomolar) at D₂ (both D_{2S} and D_{2L}) and D₃ receptors in stably transfected cell membrane

preparations. Functional activity of trazpiroben at the D_{2L} receptor was determined as G-protein activation compared with the agonist dopamine by measurement of guanosine Eu-GTP binding in fluorescence assays. Trazpiroben potently inhibited dopamine-mediated activation of the D_{2L} receptor (measured via activation of cognate G-proteins), acting as a neutral antagonist at the D_{2L} receptor. Trazpiroben was selective for D₂ and D₃ receptors, with weaker affinities at α_{1B} , 5-HT_{2A}, and D₄ receptors and negligible affinity for a panel of approximately 80 other molecular targets tested. These results indicated a receptor binding profile for trazpiroben closely resembling that of domperidone, although the two compounds have distinctly different molecular structures.

Domperidone has a relatively high degree of plasma protein binding reportedly between 92% and 93% (Barone, 1999). *In vitro* studies with trazpiroben showed a similar degree of plasma protein binding, namely 93% and 92% in the plasma of the rat and dog, respectively. For drug candidates, an important determinant of brain penetration is the degree of plasma protein binding, as only the unbound drug fraction can penetrate the BBB (Reichel, 2009). Additionally, trazpiroben is amphoteric owing to its carboxylic acid functionality. Thus, trazpiroben is expected to demonstrate low lipophilicity, high polarity, and reduced cell permeability due to the overwhelming dominance of the zwitterionic form at physiologic pH. High plasma protein binding and the compound's chemical characteristics indicate poor passive permeability reflected in the minimal brain penetration observed in the rat and dog and lack of CNS effects observed in the rat and humans, therefore suggesting that trazpiroben may potentially have an improved CNS safety profile versus more centrally penetrating D₂/D₃ antagonists like metoclopramide and may be associated with a low risk of EPS similar to domperidone (Jasper and Whiting, 2020; Whiting et al., 2021).

The CNS safety of trazpiroben was evaluated via accelerating rotarod performance of the rat after oral gavage. Trazpiroben was not associated with meaningful effects on motor coordination in the rat at any dose investigated. In comparison, the centrally acting D₂/D₃ antagonist chlorpromazine substantially reduced time spent by the rat on the accelerating rotarod after oral administration (Sokoloff et al., 1992). The lack of trazpiroben-mediated effect on accelerating rotarod performance versus effects seen with a centrally acting D₂/D₃ antagonist are consistent with the low brain penetration of

TABLE 3
Trazpiroben effects on the hERG potassium channel current.

Trazpiroben Concentration	Percentage Inhibition			Number of Cells
	Mean	S.D.	S.E.M.	
μM				
0	3.1	1.9	1.1	3
4.9	15.9 ^a	0.2	0.1	3
10.0	38.5 ^a	0.2	0.1	3
26.6	65.6 ^a	6.6	3.8	3
44.9	79.9 ^a	0.4	0.2	3

^aDifference is statistically meaningful from that of vehicle alone.

Half-maximal inhibitory concentration is 15.6 μM with a Hill coefficient of 1.3 when fitted to a curve.

trazpiroben observed in the present distribution studies in the rat and dog. Additionally, safety pharmacology studies have shown no meaningful effects on functional observation battery assessments and negligible locomotor effects of trazpiroben in the rat (Kreckler et al., 2020).

The primary pharmacology of trazpiroben was further demonstrated in established in vivo D_2 antagonism models, including effects on prolactin secretion in the rat and dog and on apomorphine-induced emesis in dog. Prolactin is primarily secreted from lactotroph cells of the anterior pituitary gland, with release under inhibitory control by dopamine (Fitzgerald and Dinan, 2008). D_2 receptor activation on lactotroph cells inhibits prolactin gene transcription and synthesis, and therefore, blockade of D_2 receptors counteracts the tonic inhibitory effect of dopamine on prolactin secretion (Freeman et al., 2000; Fitzgerald and Dinan, 2008). Trazpiroben increased prolactin release in the rat after single oral doses (0.1–10 mg/kg) and in rat and dog after multiple administrations of oral gavage solution and oral capsules at doses of 100 and 50 mg/kg, respectively. These results indicate D_2 receptor blockade in the anterior pituitary gland, which is consistent with the primary pharmacology of trazpiroben demonstrated in vitro.

Emetic response in the dog and humans is, among other factors, under the control of D_2 receptors in the CTZ of the area postrema (Welliver, 2014). Administration of the D_2/D_3 agonist apomorphine can be used to stimulate an emetic response in beagle dogs, and inhibition of this response provides an in vivo measure of D_2/D_3 antagonism in the CTZ (Levant, 1997). Trazpiroben inhibited apomorphine-induced retching/emetic episodes in dog after oral gavage with a potency (0.1–1 mg/kg) similar to that of the positive control, domperidone (Sokoloff et al., 1992). The potency of trazpiroben-mediated inhibition of emetic responses in the dog was similar to that of trazpiroben-mediated increases of prolactin release in rat. Serum prolactin level may therefore be used as a biomarker to indicate target (D_2 receptor) engagement. As both the anterior pituitary gland and the area postrema lie outside the BBB, effects on prolactin secretion in the rat and dog and on apomorphine-induced emesis in dog are therefore suitable models for demonstrating the potent D_2 antagonism of trazpiroben in vivo, despite its limited BBB penetrability.

In good laboratory practice manual patch clamp electrophysiology studies of mammalian cells, trazpiroben only weakly blocked the hERG potassium channel current ($IC_{50} = 15.6 \mu M$), representing a 10,000-fold selectivity versus affinities (1.5 nM and 3.2 nM) at target D_2/D_3 receptors. The cardiac potassium channel hERG, which is responsible for I_{Kr} in human ventricles, was evaluated because I_{Kr} inhibition is the most common cause of cardiac action potential prolongation by noncardiac drugs (Brown and Rampe, 2000; Rampe and Brown, 2013). Increased action potential duration causes QT interval prolongation and has been associated with the dangerous ventricular arrhythmia torsades de pointes (Brown and Rampe, 2000; Rampe and Brown, 2013). Domperidone, a potent inhibitor of the hERG channel, is associated with a risk of serious cardiac side effects (Michaud and Turgeon, 2013). In our studies, trazpiroben was a weak inhibitor of the hERG channel relative to domperidone, reducing the potential for fatal arrhythmias and potentially offering an improved cardiac safety profile. This is consistent with safety pharmacology studies in instrumented dog and phase 1 clinical studies that

showed no effects on corrected QT duration (Kreckler et al., 2020; Whiting et al., 2021).

In summary, the in vivo pharmacological profile of the novel, potent dopamine D_2/D_3 receptor antagonist trazpiroben is similar to that of domperidone. However, unlike domperidone, trazpiroben has a higher therapeutic safety window in terms of the potential for corrected QT prolongation. Our data suggest trazpiroben may offer potent anti-nauseant and antiemetic activity and may have a favorable safety profile. Further studies in patients with gastroparesis are ongoing to confirm the safety and efficacy profile of trazpiroben.

Authorship Contributions

Participated in research design: Whiting, Choppin, Jasper.

Conducted experiments: Choppin, Jasper.

Contributed new reagents or analytic tools: Luehr.

Performed data analysis: Whiting, Choppin, Jasper.

Wrote or contributed to the writing of the manuscript: Whiting, Choppin, Luehr, Jasper.

Note Added in Proof: A reference was accidentally included in Figure 5 caption in the Fast Forward version published July 12, 2021. Figure 5 caption has now been corrected.

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