

CSTI-300 (SMP-100); a Novel 5-HT₃ Receptor Partial Agonist with Potential to Treat Patients with Irritable Bowel Syndrome or Carcinoid Syndrome^{SI}

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ABSTRACT

The 5-hydroxytryptamine (5-HT) (serotonin) 5-HT₃ receptor represents a clinical target for antagonists to deliver symptomatic relief to patients with diarrhea-predominant irritable bowel syndrome (IBS-d) or carcinoid syndrome. Unfortunately, this pharmacological strategy can present side effects (e.g., severe constipation). The present study investigates the potential of a novel 5-HT₃ receptor partial agonist, CSTI-300, to treat patients with IBS-d and other conditions associated with discomfort from colonic distension, with a predicted reduced side-effect profile. The *in vitro* and *in vivo* preclinical pharmacology of the drug CSTI-300 was investigated to explore the potential to treat patients with IBS-d. CSTI-300 displayed selective high affinity for the human and rat 5-HT₃ receptor (K_i approximately 2.0 nM) and acted as a partial agonist (approximately 30%–50% intrinsic efficacy) *in vitro*. In an *in vivo* model of IBS-d, the rat colon distension model, CSTI-300 displayed dose-dependent efficacy. In addition, oral administration of CSTI-300 to dogs that achieved plasma levels of the drug exceeding the K_i value for the 5-HT₃ receptor failed to either evoke emesis or alter the state of feces.

Pharmacokinetics for CSTI-300 in rat and dog identified high levels of oral availability with *t*_{1/2} range of 1.6–4.4 hours. The preclinical pharmacology of the lead candidate drug, CSTI-300, supports the potential of this novel drug to offer symptomatic relief to patients with irritable bowel syndrome and carcinoid syndrome with a rationale for a reduced “on-target” side-effect profile relative to 5-HT₃ receptor antagonists, such as alosetron.

SIGNIFICANCE STATEMENT

There is a lack of effective current treatment for diarrhea-predominant irritable bowel syndrome and carcinoid syndrome, and in both conditions, overactivity of the 5-hydroxytryptamine (5-HT) 5-HT₃ receptor is thought to be implicated in the pathophysiology. Because 5-HT₃ receptor blockade with antagonists results in significant side effects, we present evidence that treatment with a suitable 5-HT₃ receptor partial agonist will alleviate some symptoms associated with these conditions yet, without fully inhibiting the receptor, predict a less pronounced side-effect profile associated with this therapeutic strategy.

Introduction

Irritable bowel syndrome (IBS) is a defined functional bowel disorder (Rome IV criteria; e.g., Drossman, 2016; Drossman

and Hasler, 2016) that is often debilitating and sometimes presents severe abdominal pain (Lacy and Moreau, 2016) with colonic distension. Prevalence is around 10% to 11%, with a female:male ratio of 2:1 (Longstreth et al., 2006; Pleis and Lucas, 2009; Enck et al., 2016; Quigley et al., 2016). With comorbidities, including anxiety/depression (e.g., Fond et al., 2014), IBS contributes a considerable burden upon the health-care system and loss of societal productivity (annual societal costs above \$21 billion in the United States; <https://www.aboutibs.org/facts-about-ibs/statistics.html>; Ashburn and Gupta, 2006; Buono et al., 2017).

IBS is subcategorized into four forms based on presenting clinical symptoms (Enck et al., 2016): diarrhea-predominant (IBS-d), constipation-predominant (IBS-c), alternating symptoms

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Conflicts of interest. D.X. and J.H. are executives of Chengdu SciMount Pharmatech Co. Ltd. S.L. is an executive of ConSynance Therapeutics, Inc. P.R.G. was an employee of Albany Molecular Research, Inc. and an executive of ConSynance Therapeutics, Inc. and is currently an advisor to Chengdu SciMount Pharmatech Co. Ltd. D.D.M., N.A.M., and B.J.S. are current/former employees of Albany Molecular Research, Inc. N.M.B. was a former consultant of Albany Molecular Research, Inc. and an advisor to ConSynance Therapeutics, Inc. and Chengdu SciMount Pharmatech Co. Ltd.

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between diarrhea and constipation (IBS-mixed), and unsubtyped IBS. IBS-d represents approximately one-third of the IBS patient population (Pleis and Lucas, 2009; Quigley et al., 2016).

Despite the substantial clinical need, the market for effective therapies for IBS is considered naïve and underpenetrated. There are no pharmaceuticals specifically indicated for IBS-mixed and just two products with IBS-c as an indication that often deliver insufficient benefit and/or are prone to side effects and are hence considered second-line treatments subsequent to failure of laxatives/diet adjustments, which themselves have very limited success (Camilleri and Boeckxstaens, 2017; Craig, 2018).

There is no established well-tolerated treatment of IBS-d, and common remedies (diet adjustments and fiber regimens) have very limited success. Within the United States, Allergan's opiate receptor agonist, eluxadoline (Viberzi), was introduced, recently facilitated by Food and Drug Administration (FDA) "Fast Track" status, yet displays limited efficacy and is associated with common side effects, such as constipation and nausea as well as a report of ischemic colitis (Lacy and Moreau, 2016; Fragkos, 2017); moreover, some patients are at risk of developing pancreatitis (Camilleri, 2017), resulting in a warning from the FDA (<https://www.fda.gov/Drugs/DrugSafety/ucm546154.htm>). Another drug, the opioid receptor agonist ORP-101, also received "Fast Track" status (<http://www.orphomed.com/newsroom/press-releases/2018-04-25/>), demonstrating the continued recognition of the unmet therapeutic need.

It has been known for over 2 decades that 5-HT₃ receptor antagonists, such as alosetron, deliver strong clinical efficacy to patients with IBS-d (e.g., Crowell, 2004), presumably by blocking elevated 5-HT function in these patients (Bearcroft et al., 1998; Dunlop et al., 2005; Enck et al., 2016; Fu et al., 2019; Gunn et al., 2019) that may arise from lower expression of the 5-HT transporter (Faure et al., 2010) to decrease gut motility and visceral sensation (e.g., Michel et al., 2005; Kapeller et al., 2011). Furthermore, increased expression of gut 5-HT₃ receptor by patients with IBS-d (Gunn et al., 2019) and associated 5-HT₃ receptor subunit polymorphisms (Kapeller et al., 2008, 2009; Kilpatrick et al., 2011; Gunn et al., 2019) support therapeutic targeting of this receptor.

However, patients with IBS-d receiving 5-HT₃ receptor antagonists often report (potentially severe) constipation, and rare instances of ischemic colitis are evident (around 1:750 patients; see Zheng et al., 2017), which led to the withdrawal of the marketing authorization for alosetron by the FDA and may have contributed to the decision to stop clinical development of cilansetron (issues of severe constipation and ischemic colitis were also evident; Chey and Cash, 2005). Unusually, the FDA subsequently reinstated the marketing authorization for alosetron, albeit initially with a "black box" warning. Upon reflection, it would appear that high levels of 5-HT₃ receptor inhibition are likely responsible for the severe constipation and possibly ischemic colitis. However, the latter side effect is likely a consequence of a multifactorial mechanism that includes the presence of IBS-d itself, since unmedicated patients can present this symptom

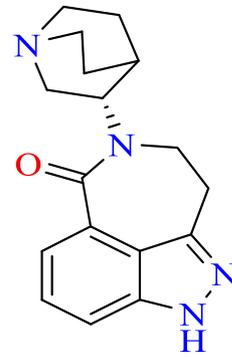


Fig. 1. The structure of CSTI-300 (also known as SMP-100).

and non-IBS-d patients receiving 5-HT₃ receptor antagonists for other indications (e.g., emesis), including at relatively high dosage, do not present ischemic colitis (Tricco et al., 2016).

Knowledge of the pathophysiology of IBS-d forward an opportunity of a pharmacological mechanism predicted to deliver clinical efficacy with a reduced side-effect profile. Thus, a selective 5-HT₃ receptor *partial* agonist would reduce receptor activity evoked by endogenous 5-HT yet would prevent complete receptor inhibition reducing the likelihood of constipation and perhaps ischemic colitis. A similar rationale is relevant to offer symptomatic relief to patients with carcinoid syndrome, which is associated with copious quantities of circulating 5-HT with arising chronic diarrhea (e.g., Davis et al., 1973; Kvols, 1994; Halperin et al., 2017) that can be controlled by the "off-label" use of 5-HT₃ receptor antagonists (Anderson et al., 1987; Jacobsen, 1992; Platt et al., 1992; Schworer et al., 1995; Wymenga et al., 1998; Kiesewetter and Raderer, 2013; Halperin et al., 2017). The present report describes the preclinical pharmacology of the lead candidate novel 5-HT₃ receptor partial agonist, CSTI-300 (Fig. 1), and the potential of the drug to treat some of the symptoms of IBS-d and carcinoid syndrome with a predicted reduced side-effect profile relative to 5-HT₃ receptor antagonists like alosetron.

Materials and Methods

Nomenclature. The 5-HT₃ receptor nomenclature conforms to international guidelines (Alexander et al., 2015).

Cell Culture

Human embryonic kidney 293 (HEK293) cells stably expressing the human functional 5-HT_{3A} receptor (HEK293-h5-HT_{3A} cells, Dubin et al., 1999; Brady et al., 2001) were cultured in Dulbecco's modified Eagle's medium [supplemented with 10% (v/v) FBS, 1.0% (v/v) penicillin/streptomycin (100 U/ml penicillin and 0.1 mg/ml streptomycin), and the selection antibiotic, G418 (250 µg/ml)] and maintained at 37°C, 5% CO₂, and 95% air at 95% relative humidity. The same conditions were used for cells stably expressing the human 5-HT_{3AB} receptor (HEK293-h5-HT_{3AB}), except the media was also supplemented with Zeocin (80 µg/ml) as well as G418 (Brady et al., 2001). HEK293 cells stably expressing the rat functional 5-HT_{3A} receptor (HEK293-r-5-HT_{3A}) were cultured the same as for HEK293-hour5-HT_{3A} cells.

ABBREVIATIONS: AUC, area under the curve; Cl-indole, 5-chloroindole; FDA, Food and Drug Administration; HEK293, human embryonic kidney 293; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; IBS, irritable bowel syndrome; IBS-c, constipation-predominant IBS; IBS-d, diarrhea-predominant IBS; p.o., per os; UPLC, ultra-performance liquid chromatography; Vd, volume of distribution.

Radioligand Binding. Radioligand binding assays were performed as described by us previously (e.g., Monk et al., 2004). Briefly, washed HEK293-h5-HT₃A, HEK293-h5-HT₃AB, or HEK293-r5-HT₃A cells were homogenized (Polytron) in 25 mM Tris buffer (pH 7.4). For competition assays, individual binding assays were performed in triplicate, and binding tubes contained 100 μ l of competing drug(s) or vehicle (Total binding; Tris buffer) and 100 μ l of [³H]-granisetron (~0.5 nM; ~3.0 TBq/mmol; Perkin-Elmer, Seer Green, UK) before cell homogenate (100 μ l) was added to initiate binding, which proceeded at room temperature for 60 minutes before termination by rapid filtration (Whatman GF/B filter) and subsequent washing (ice-cold Tris buffer) under vacuum through Whatman GF/B filters, followed by assay of the radioactivity remaining on the filters. For saturation binding assays, to demonstrate that CSTI-300 is a competitive ligand at the h5-HT₃A and h5-HT₃AB receptors, a range of [³H]-granisetron concentrations (0.1–30 nM) were used in the absence (vehicle) or presence of CSTI-300, with ondansetron (10 μ M) used to define nonspecific binding, with each condition performed in duplicate. Cell homogenate samples were then incubated and harvested as for competition assays.

[Ca²⁺]_i Assays. The day before performing intracellular calcium assays, HEK293-h5-HT₃A, HEK293-h5-HT₃AB, or HEK293-r5-HT₃A cells were seeded at a density of 1×10^5 cells per well into black-sided, clear-bottomed 96-well plates (Sigma-Aldrich, Poole, UK). On the day of assay, cells were washed twice with $1 \times$ Hanks' balanced salt solution (pH 7.4; Life technologies, Paisley, UK) and incubated with fluo-4 acetoxymethyl ester (5.0 μ M; Life technologies, Paisley, UK) for 1 hour at room temperature. Cells were then washed twice with Hanks' balanced salt solution and incubated for a further 30 minutes prior to assay (Newman et al., 2013). [Ca²⁺]_i was measured using a Flex station (Molecular Devices, Sunnyvale, CA) with fluorescence recorded every 3 seconds. At 80 seconds, 5-HT or CSTI-300 was applied rapidly to cells. At 320 seconds, ionomycin (750 μ g/ml) was added to demonstrate that the cells were still able to elicit an [Ca²⁺]_i response at the end of the assay. For studies with the 5-HT₃ receptor antagonist granisetron (500 nM), the drug was applied during the 30-minute incubation period before initiation of the [Ca²⁺]_i measurements and remained present for the period of experimentation.

Single-Cell Electrophysiology. Approximately 18 hours prior to electrophysiology assays, HEK293-h5-HT₃A cells were seeded directly onto 13-mm diameter glass coverslips coated with poly-L-lysine and fibronectin at a density of 2×10^4 HEK293-5-HT₃A cells per coverslip. Macroscopic currents were recorded in the whole-cell recording mode of the patch-clamp technique. Cells were superfused at 2.0 ml/min with an extracellular solution composed of 140 mM NaCl, 2.8 mM KCl, 1.0 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.4, adjusted with NaOH. Patch electrodes were pulled from borosilicate glass (o.d. 1.2 mm, i.d. 0.69 mm; Harvard Apparatus, Edenbridge, UK) using a P-97 puller (Sutter, Novato, CA) and filled with intracellular solution consisting of 140 mM CsCl, 2.0 mM MgCl₂, 10 mM HEPES, 1.0 mM EGTA, 1.0 mM Mg-ATP, and 0.3 mM Na-GTP; pH was adjusted to 7.3 with CsOH (osmolarity ~285 mOsm). Patch electrodes typically had open tip resistances of 4–7 M Ω . Membrane currents were recorded using an Axopatch 200B amplifier (Molecular Devices, Wokingham, UK), low-pass Bessel-filtered at 1.0 kHz, and digitized at 10 kHz by a Digidata 1550B (Molecular Devices). Experiments were performed at room temperature with the cells voltage-clamped at –60 mV.

Agonist-evoked currents were elicited by pressure ejection (1.3 bar; Picospritzer III; Parker Hannifin, Pine Brook, NJ) of agonist from patch pipettes placed ~30 μ m from the recorded cell.

Rat Colon Distension Model of IBS-d. A previously described IBS-d model (Banner and Sanger, 1995; Banner et al., 1995) was used to evaluate the activity of CSTI-300 versus alosetron. This *in vivo* model applies a noxious colorectal distension to conscious rats by acute balloon inflation and the effects observed as abdominal muscle contraction, with the threshold pressure noted as the readout. The protocol complied with and was approved by the Institutional Animal Care and Use Committee (Shanghai Medicilon Inc., Shanghai, China),

and Shanghai Medicilon Inc. is accredited by the National Institutes of Health Office of Laboratory Animal Welfare (<https://olaw.nih.gov/home.htm>) and Association for Assessment and Accreditation of Laboratory Animal Care (www.aaalac.org). Rats had access to food and water *ad libitum* in a temperature-controlled environment (12-hour light/dark cycle). Prior to the experiment, rats were fasted overnight.

Given the known ability of isoflurane and some other volatile anesthetics to activate/sensitize transient receptor potential vanilloid 1 channels (Cornett et al., 2008) that would likely complicate the sensory readout from the IBS-d model, ether was selected as the anesthetic agent. Thus, under ether anesthesia, a 6- and 7-cm long latex balloon was carefully inserted intra-anally to a position ~1 cm beyond the anorectal verge. The cannula from the balloon, which was taped to the tail to prevent expulsion of the balloon, was connected to a colored water-filled manometer and via a three-way tap to a syringe pump. Throughout the procedure, the singly housed animals (male Wistar rats; 180–250 g) were allowed unrestricted movement within a 20 \times 20 \times 14-cm clear acrylic box.

After recovery from the balloon insertion procedure, a ramp inflation of the colorectal balloon at a rate of 20 mm water per minute was performed until the visceromotor response (abdominal muscle contraction) was observed. At this point, the pressure was noted and released immediately by opening the three-way tap. The inflation procedure was repeated at 5-minute intervals until three stable responses were recorded.

Rats were then dosed with 5-hydroxytryptophan (5-HTP) (10 mg/kg) subcutaneously, and 5 minutes later a ramp inflation of the colorectal balloon was performed until the visceromotor response was observed. The inflation procedure was repeated at 5-minute intervals until three stable responses were recorded before subsequent treatment; either alosetron (0.01–1.0 mg/kg; 1.0 ml/kg *s.c.*), CSTI-300 (0.01–1.0 mg/kg; 1.0 ml/kg *s.c.*), or vehicle (0.9% NaCl; 1.0 ml/kg *s.c.*). The colonic distensions were performed at 5-minute intervals for a further 45 minutes with effective pressures monitored.

Dog Behavioral and Emesis Model. The protocol for the dog behavioral and emesis model complied with and was approved by the Institutional Animal Care and Use Committee (Shanghai Medicilon Inc.). Dogs were housed individually with access to food and water *ad libitum* in a temperature-controlled environment (18–29°C; relative humidity: 30%–70%; 12-hour light/dark cycle). Prior to the experiment, dogs were fasted for 30 minutes prior to CSTI-300 administration and for 30 minutes post-CSTI-300 administration.

A total of six naïve male Beagle dogs (10.15–11.45 kg) were administered CSTI-300 (1.0 mg/kg; 5.0 ml/kg *p.o.*) or vehicle (sterile water, 5.0 ml/kg *p.o.*). Each animal was monitored continuously (by an observer and a video recording) for least 4 hours post-treatment (which would have been extended up to 6 hours if any remarkable observations had been evident during the first 4 hours postdose). During the observation period, each animal was observed primarily for signs of behavioral disturbances or emesis. Emesis was defined by retching and vomiting; “retch” was defined as the action, and “vomit” was defined as the delivery of gastric content through the retching action. Whether either or both of these emetic characteristics had occurred, they would have been counted independently and recorded on a raw data sheet. Observations for other adverse events were also performed [e.g., abdominal contractions, excessive salivation, state of fecal matter (e.g., normal, loose, diarrhea), general distress, vocalizing (e.g., excessive barking or whimpering), atypical behavior, atypical feeding behavior, atypical drinking behavior, indication of pain, or mortality]. With no emesis or adverse observations, the observation at the time point was noted as “normal.” Animals were also observed for 1 hour at 23 to 24 hours postdose, and the above observations were assessed. Whether any emesis or any adverse observation had been evident, the animals would have been monitored periodically until normal behavior was resumed (with the above observations monitored and recorded).

Six hours after treatment, a peripheral venous blood sample was taken (~1 ml/sample into sodium heparin collection tubes) from each animal for bioanalysis of the plasma concentrations of CSTI-300 by high-performance liquid chromatography tandem mass spectrometry. Briefly; plasma samples (30 μ l) were mixed with 150 μ l 0.1% formic

acid in water and transferred to a 96-well plate for injection. The liquid chromatography system was comprised of a Waters Ultra-Performance Liquid Chromatography (UPLC) (Waters Corporation) equipped with an ACQUITY UPLC binary solvent manager, ACQUITY UPLC Autosampler Module, ACQUITY UPLC sample organizer and ACQUITY UPLC column heater HT. Samples (3.0 μ l) were injected onto an ACQUITY UPLC BEH C18 1.7- μ m (50 \times 2.10 mm) column, and the mobile phase was run at 600 μ l/min. Postcolumn mass spectrometric analysis was performed using an API 4000 (triple-quadrupole) instrument from Applied Biosystems/MDS Sciex with an ESI Ionsource. The data acquisition and control system were created using Analyst 1.5.1 Software from Applied Biosystems/MDS Sciex. A calibration curve using CSTI-300 was created to enable quantification of CSTI-300 levels in the plasma.

Pharmacokinetic Studies. The pharmacokinetics of CSTI-300 was investigated in male Sprague-Dawley rats (288–322 g) and male Beagle dogs (8.0–12 kg).

Drug administration to rats was performed by NoAb BioDiscoveries (Mississauga, Ontario, Canada) after approval of procedures by the NoAb BioDiscoveries animal care committee and performed in accordance with the principles of the Canadian Council on Animal Care. Rats were housed two per cage with access to food and water ad

libitum in a temperature-controlled environment (12-hour light/dark cycle). Prior to the experiment, rats were fasted overnight and for 2 hours post-CSTI-300 administration.

Drug administration to dogs was performed by Covance Laboratories Inc. (Madison, WI) and performed in compliance with the Animal Welfare Act Regulations (9 CFR 3). Dogs were housed individually with access to food and water ad libitum in a temperature-controlled environment (12-hour light/dark cycle). Prior to the experiment, dogs were fasted overnight and for 4 hours post-CSTI-300 administration.

CSTI-300 was administered either intravenously or orally (p.o) in sterile water as vehicle, with blood samples taken at predetermined times into K_2 EDTA anticoagulant tubes. For oral studies, the dose volumes were 5.0 and 2.0 ml/kg for rat and dog, respectively. For intravenous studies, the dose volumes were 1.0 and 2.0 ml/kg for rat and dog, respectively. Plasma samples underwent protein precipitation with acetonitrile containing 0.1% formic acid. After sonication for 1 minute followed by vortex mixing for 2 minutes and refrigeration for 30 minutes, supernatants were transferred to a 96-well filter plate and filtered in a centrifuge. Filtered extracts (200 μ l) were transferred into separate 96-well plates and blown dry under nitrogen at 40°C. The residue was reconstituted in 100 μ l of mobile phase and submitted for

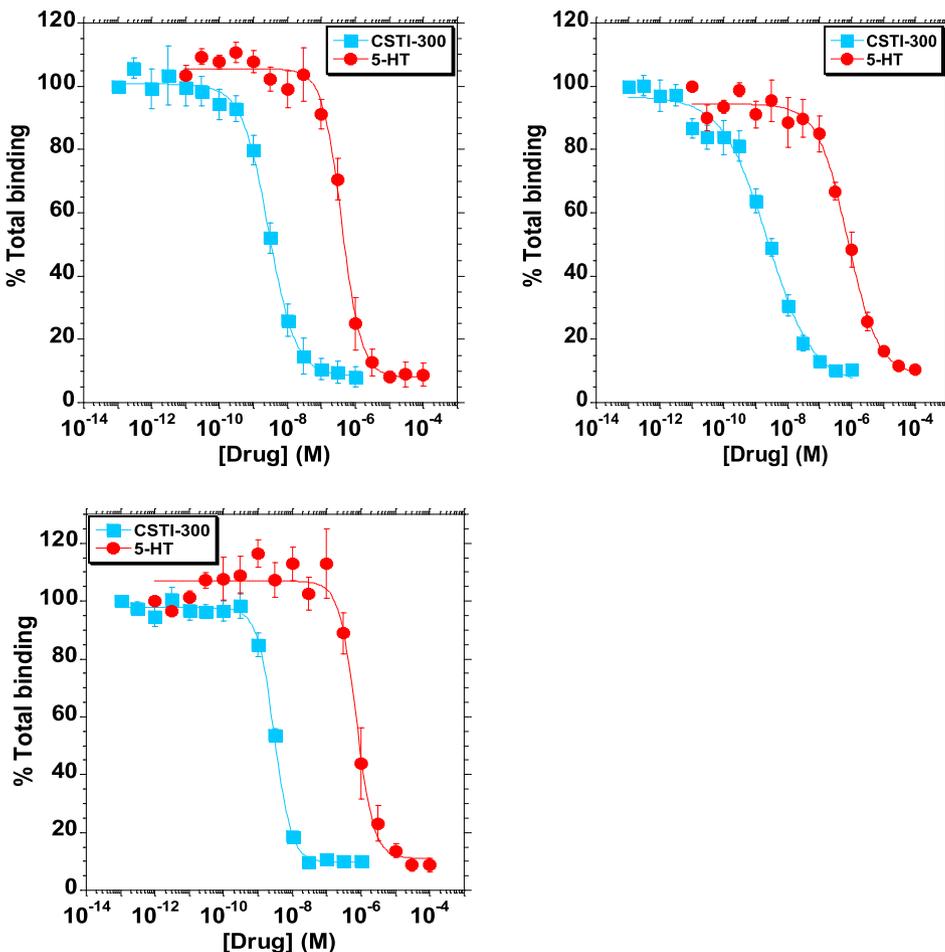


Fig. 2. The affinity of CSTI-300 for h5-HT₃A, h5-HT₃AB, and r5-HT₃A receptors. The ability of CSTI-300 or 5-HT to compete for [³H]-granisetron binding to h5-HT₃A (top left), h5-HT₃AB (top right), or r5-HT₃A (bottom left) receptors stably expressed in HEK293 cells. Data represent mean \pm S.E.M., $n = 5$. Table insert; arising quantitative pharmacological data for CSTI-300 or 5-HT for the h5-HT₃A, h5-HT₃AB, and r5-HT₃A receptors determined by radioligand binding. Data represent mean \pm S.E.M., $n = 5$.

Drug	h5-HT ₃ A receptor		h5-HT ₃ AB receptor		r5-HT ₃ A receptor	
	K _i (nM)	Hill number	K _i (nM)	Hill number	K _i (nM)	Hill number
CSTI-300	2.26 \pm 0.48	1.19 \pm 0.16	1.59 \pm 0.09	0.70 \pm 0.13	2.36 \pm 0.04	1.89 \pm 0.26
5-HT	327 \pm 62	1.49 \pm 0.19	563 \pm 89	0.91 \pm 0.07	740 \pm 237	2.05 \pm 0.27

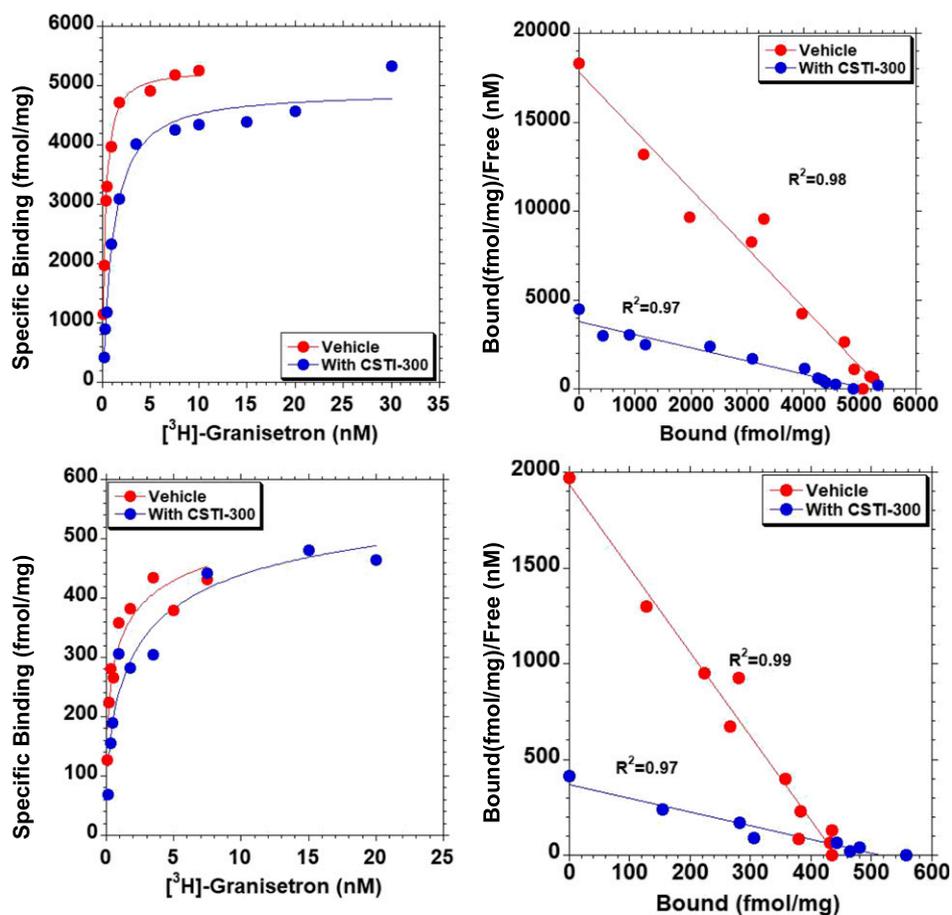


Fig. 3. CSTI-300 is a competitive ligand at the h5-HT_{3A} and h5-HT_{3AB} receptors. The impact of CSTI-300 upon the apparent affinity of specific [³H]-granisetron for h5-HT_{3A} (± 3.0 nM CSTI-300; upper panels; $P < 0.05$, Mann-Whitney U test) and h5-HT_{3AB} receptors (± 2.0 nM CSTI-300; lower panels; $P < 0.05$, Mann-Whitney U test), although there was no significant effect on labeled receptor density in either receptor preparation. Left panels: Representative saturation isotherm. Right panels: Scatchard transformation of the saturation isotherm. Specific binding = total binding – non-specific binding detected in the presence of ondansetron (10 μ M). Table insert: arising quantitative pharmacological data. Data represent mean \pm S.E.M., $n = 5$.

Treatment	h5-HT _{3A} receptor			h5-HT _{3AB} receptor		
	K _d (nM)	B _{max} (fmol/mg)	Hill number	K _d (nM)	B _{max} (fmol/mg)	Hill number
Vehicle	0.38 \pm 0.05	3414 \pm 582	1.16 \pm 0.10	0.95 \pm 0.21	769 \pm 83	0.66 \pm 0.06
CSTI-300	1.42 \pm 0.19	3264 \pm 552	1.52 \pm 0.48	3.40 \pm 0.84	845 \pm 159	0.83 \pm 0.06

liquid chromatography with tandem mass spectrometry analysis. CSTI-300 was quantified with Analyst 1.4.2 software using a calibration curve. Plasma concentration data were analyzed with WinNonlin 5.2 software using a noncompartmental model to calculate pharmacokinetic parameters.

Data and Statistical Analysis. Values are expressed as mean \pm S.E.M. Curve-fitting data analysis was performed with KaleidaGraph (version 3.5). Two-way ANOVA with subsequent Tukey's multiple comparisons test (with Bonferroni correction for multiple comparisons) were used to evaluate potential treatment differences in the rat IBS-d colon model; a Shapiro-Wilk procedure tested normality. A Kruskal-Wallis test with Dunn's multiple comparisons was used to test any differences in 5-chloroindole (Cl-indole) affinity for the h5-HT_{3A} receptor and any differences between CSTI-300 and vehicle treatment in rat IBS-d colon model. A Mann-Whitney U test was used to determine whether the impact of CSTI-300 upon [³H]-granisetron affinity for the h5-HT₃ receptor was significant. Post hoc tests were only conducted if P was < 0.05 and the variance of the data was homogenous. Significance criterion was $P < 0.05$. Data and statistical analysis complied with the recommendations

on experimental design and analysis in pharmacology (Curtis et al., 2015).

Results

Competition Radioligand Binding. CSTI-300 and 5-HT competed for [³H]-granisetron binding to the h5-HT_{3A}, h5-HT_{3AB}, or r5-HT_{3A} receptors, with CSTI-300 demonstrating over 100-fold greater affinity compared with 5-HT (Fig. 2). The affinity of CSTI-300 was comparable at the h5-HT_{3A}, h5-HT_{3AB}, and r5-HT_{3A} receptors. The Hill number for CSTI-300, like 5-HT, was above unity when competing for the h5-HT_{3A} or r5-HT_{3A} receptors (Fig. 2). However, this was not the case when either CSTI-300 or 5-HT were competing for the h5-HT_{3AB} receptor (Fig. 2).

The endogenous agonist 5-HT, unlike 5-HT₃ receptor antagonists, has been shown previously to reveal an impact of the cryptic orthosteric modulator Cl-indole, allowing Cl-indole to

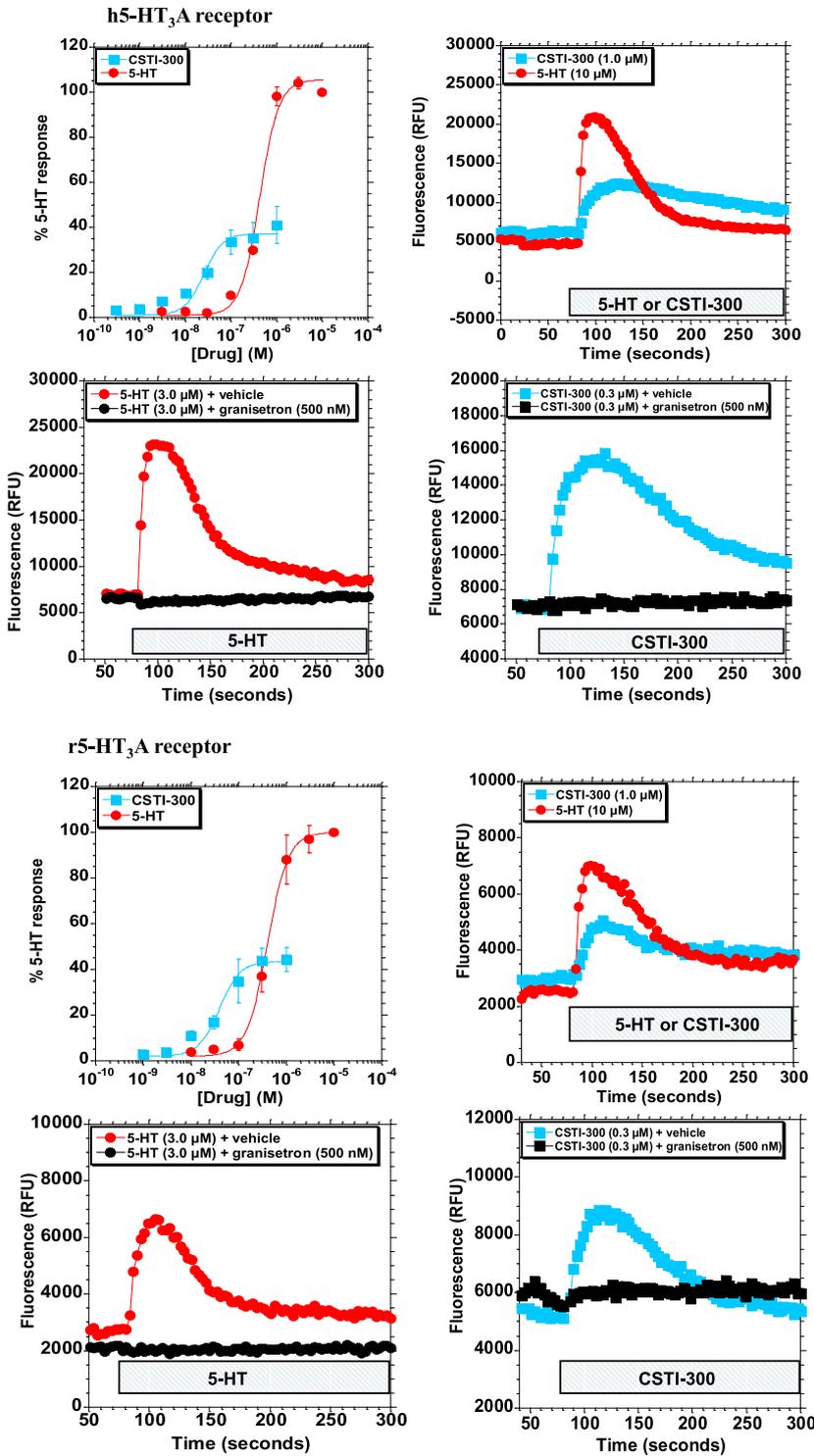


Fig. 4. CSTI-300 is a partial agonist at the h5-HT_{3A} and r5-HT_{3A} receptor. Ability of CSTI-300 to activate the h5-HT_{3A} (upper four panels; h5-HT_{3A}) or r5-HT_{3A} (lower four panels; r5-HT_{3A}) receptor assessed by an increase in [Ca²⁺]_i in HEK293 cells stably expressing the h5-HT_{3A} or r5-HT_{3A} receptor. Top left: concentration-dependent action of CSTI-300 or 5-HT (mean ± S.E.M., n = 4 to 5). Top right: representative traces displaying change in fluorescence over time prior to and subsequent to the administration of CSTI-300 (1.0 μM) or 5-HT (10 μM). Bottom panels: representative traces (n = 4 to 5) displaying change in fluorescence over time prior to and subsequent to the administration of 5-HT (3.0 μM) or CSTI-300 (0.3 μM) in the absence (vehicle) or presence of granisetron (500 nM; 30 minutes pretreatment). Table insert: arising quantitative pharmacological data. Data represent mean ± S.E.M., n = 4 to 5.

Drug	h5-HT _{3A} receptor			r5-HT _{3A} receptor		
	E _{max}	EC ₅₀ (nM)	Hill number	E _{max}	EC ₅₀ (nM)	Hill number
CSTI-300	41±7	25±5	0.85±0.14	51±3	56±17	1.14±0.21
5-HT	104±1	400±17	3.28±0.46	102±3	407±52	2.83±0.93

compete for [³H]-granisetron binding to the h5-HT_{3A} receptor (Powell et al., 2016). CSTI-300, like 5-HT (see also Powell et al., 2016), revealed in a concentration-dependent manner

(1.0–5.0 nM) the ability of Cl-indole to compete for [³H]-granisetron binding to the h5-HT_{3A} receptor (Supplemental Fig. 1).

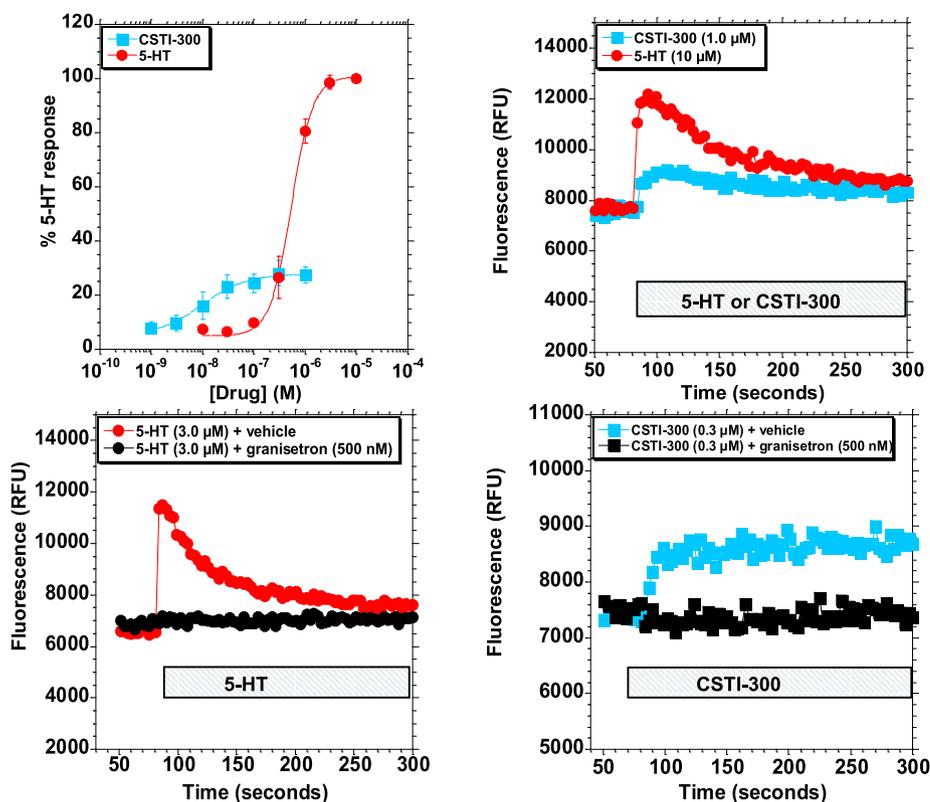


Fig. 5. CSTI-300 is a h5-HT₃AB receptor partial agonist. Ability of CSTI-300 to activate the h5-HT₃AB receptor assessed by an increase in [Ca²⁺]_i in HEK293 cells stably expressing the h5-HT₃AB receptor. Top left: concentration-dependent action of CSTI-300 or 5-HT (mean ± S.E.M., *n* = 5). Top right: representative traces displaying change in fluorescence over time prior to and subsequent to the administration of CSTI-300 (1.0 μM) or 5-HT (10 μM). Bottom panels: representative traces (*n* = 5) displaying change in fluorescence over time prior to and subsequent to the administration of 5-HT (3.0 μM) or CSTI-300 (0.3 μM) in the absence (vehicle) or presence of granisetron (500 nM; 30 minutes pretreatment). Table insert: arising quantitative pharmacological data. Data represent mean ± S.E.M., *n* = 5.

Drug	E _{max}	EC ₅₀ (nM)	Hill number
CSTI-300	30±4	12±2	0.83±0.17
5-HT	101±1	525±71	2.30±0.17

Saturation Radioligand Binding. To determine the nature of the interaction between CSTI-300 and the h5-HT₃A or h5-HT₃AB receptors, saturation radioligand binding studies using [³H]-granisetron were undertaken (Fig. 3). The presence of CSTI-300 (2.0–3.0 nM) reduced the affinity of [³H]-granisetron for both the h5-HT₃A receptor and h5-HT₃AB receptor without impacting the density of labeled receptors, indicating a competitive interaction with each receptor isoform.

Intracellular [Ca²⁺] Assays. CSTI-300 and 5-HT both activated the h5-HT₃A, h5-HT₃AB, and r5-HT₃A receptors in a concentration-dependent manner to elicit an increase in intracellular Ca²⁺ concentration in populations of HEK293 cells stably expressing the h5-HT₃A, h5-HT₃AB, or r5-HT₃A receptors *in vitro* (Figs. 4 and 5). At the h5-HT₃A receptor, CSTI-300 was ~16 times more potent than 5-HT and behaved as a partial agonist with an intrinsic efficacy of ~40%. At the h5-HT₃AB receptor, CSTI-300 displayed intrinsic activity of ~30% and was over 40 times more potent than 5-HT. At the r5-HT₃A receptor, CSTI-300 was ~7 times more potent than 5-HT and behaved as a partial agonist with an intrinsic efficacy of ~50%. Preincubation with the selective 5-HT₃ receptor antagonist, granisetron (500 nM), prevented the intracellular Ca²⁺ responses to a maximal concentration of either CSTI-300 or 5-HT, further demonstrating the

specificity of the responses via the h5-HT₃A, h5-HT₃AB, or r5-HT₃A receptors.

Electrophysiology Studies. To further elucidate the effect of CSTI-300 at the h5-HT₃A receptor, single-cell electrophysiology was performed with HEK293 cells stably expressing the h5-HT₃A receptor. At a holding potential of -60 mV, a 60-second application of a maximal 5-HT concentration (10 μM) elicited an inward current of 1256 ± 231 pA (*n* = 5; Fig. 6). The time constant of the rising phase was 0.28 ± 0.07 seconds (*n* = 5), which decayed slowly back to baseline. The decay was best modeled by a two-exponential curve fit with time constants of 57.6 ± 26.5 and 245.8 ± 50.3 milliseconds (*n* = 5). In contrast, a 60-second application of a maximal concentration of CSTI-300 (100 nM) elicited a smaller inward current (248 ± 74 pA; *n* = 9; Fig. 6) with a slower rise time (3.64 ± 0.29 seconds; *n* = 9) and decay time (one-exponential fit; 260.9 ± 31.9 milliseconds; *n* = 9) when compared with 5-HT.

Rat IBS-d Model. To demonstrate the potential of CSTI-300 to reduce colonic sensitivity (a symptom associated with IBS-d), an *in vivo* rat model of IBS-d based on published work was used (Banner and Sanger, 1995; Banner et al., 1995). The sensitivity of the conscious rats to colon distension was increased by subcutaneous application of 5-HTP (10 mg/kg s.c.; Fig. 7), the biochemical precursor to serotonin. Consistent

with previous studies, this 5-HTP-induced increased sensitivity of the colon was prevented by a selective 5-HT₃ receptor antagonist (alosepron) and also by CSTI-300 in a dose-dependent manner ($P < 0.01$ to < 0.0001). In addition, a maximal effective dose of CSTI-300 displayed similar actions to a maximal effective dose of alosetron in this in vivo model (Fig. 7), and an ED₅₀ of 0.55 mg/kg was calculated for CSTI-300.

Dog Behavioral and Emesis Study. CSTI-300 (1.0 mg/kg, p.o.) did not induce emesis in naïve dogs (Table 1). In addition, CSTI-300 did not alter the state of the feces nor the general behavior of the dogs. Six hours after treatment of the dogs with CSTI-300 (1.0 mg/kg, p.o.), the concentration of CSTI-300 in plasma was found to be between 31 and 227 nM ($n = 6$). The plasma protein binding in dog plasma was determined to be $30\% \pm 3\%$. Therefore, the free drug concentrations achieved in this study were between 10 and 70 times the K_i for CSTI-300 at the h5-HT₃ receptor, indicating that 5-HT₃ receptor-saturating concentrations of CSTI-300 were achieved through the course of the investigation.

Pharmacokinetic Studies. The pharmacokinetics of CSTI-300 were evaluated in rat and dog (Fig. 8; Table 2). After intravenous administration of CSTI-300 (1.0 mg/kg), the $t_{1/2}$ was comparable between rat (1.6 ± 0.2 hours; $n = 3$) and dog (1.6 ± 0.2 hours; $n = 4$). The volume of distribution values was moderate in the two species evaluated (3.0–14.6 l/kg). The clearance values were also moderate with somewhat lower clearance values apparent in the larger species: dog (1.3 l/h per kilogram) versus rat (6.2 l/h per kilogram). The $t_{1/2}$ for CSTI-300 was longer after oral administration and ranged from 2.3 to 4.4 hours in rat and 1.6 to 3.7 hours in dog (Fig. 8; Table 2). The oral bioavailability was good in rat (24%–41%F) and excellent in dog (89%–100%F). Increased doses of orally administered CSTI-300 trended toward supraproportional exposure [area under the curve (AUC)] within the species examined. After oral administration, the C_{max} and AUC are significantly increased for matched or similar doses in the larger animals (dog) versus the rodent species (rat).

CSTI-300 Has Desirable Pharmaceutical Characteristics. CSTI-300 displayed a variety of characteristics consistent with it being a suitable lead candidate drug for development (Table 3). Thus, CSTI-300 displayed a high level of pharmacological selectivity for the 5-HT₃ receptor, with no significant off-target activity detected (affinity $< < 1 \mu\text{M}$ for a range of neurotransmitter receptors and ion channels; Supplemental Table 1). The water solubility was excellent, and there was no need for any special formulation for in vivo work; sterile water or saline was used for all animal studies. The LogP of 1.95 is within the optimal historic range for orally active marketed drugs (Leeson and Young 2015). In a 7-day dose range finding toxicity study in rat, the no observed adverse effect level was determined to be 300 mg/kg per day, 300 times the effective dose observed in the rat colon distension model. CSTI-300 did not inhibit any of the common cytochrome 450-metabolizing enzymes, predicting a low probability for drug/drug interactions. Moreover, CSTI-300 was well-tolerated and demonstrated good oral pharmacokinetics in rodent and dog.

Discussion

This study presents the results of a detailed pharmacological investigation of the 5-HT₃ receptor partial agonist CSTI-300,

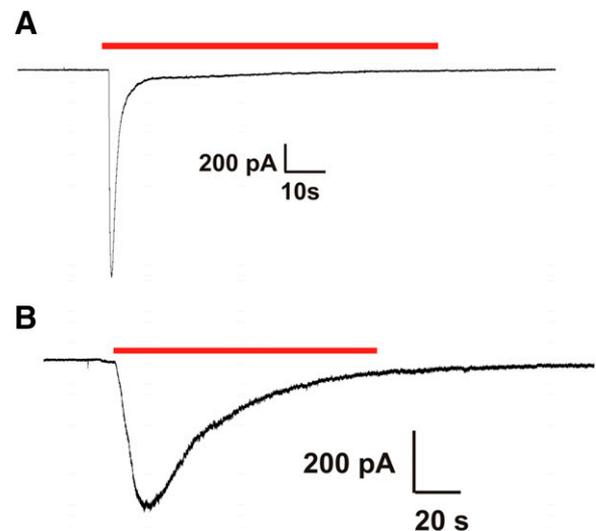


Fig. 6. Kinetics of CSTI-300 mediated activation of the h5-HT₃A receptor in comparison with 5-HT. A maximal concentration of 5-HT [10 μM , (A)] or CSTI-300 [100 nM, (B)] elicited an inward current in HEK293 cells stably expressing the h5HT₃A receptor. Drugs were applied for 60 seconds (red bar) using a Picospritzer. Data are representative of 5 (5-HT) or 9 (CSTI-300) separate experiments.

using both in vitro and in vivo models to demonstrate the potential therapeutic effectiveness of this drug as a treatment of symptoms associated with IBS-d and carcinoid syndrome (i.e., intermittent abdominal pain from bloating and gut distension).

The in vitro experiments demonstrated that CSTI-300 is a competitive and selective ligand for the h5-HT₃ receptor with relatively high affinity for the h5-HT₃A, h5-HT₃AB, and r5-HT₃A receptors in radioligand binding assays. CSTI-300 displayed partial agonist activity at the h5-HT₃A, h5-HT₃AB, and r5-HT₃A receptors, with similar properties to the orthosteric endogenous agonist, 5-HT, albeit with a lower intrinsic efficacy. Relative to the marketed 5-HT₃ receptor antagonists, ondansetron and alosetron, CSTI-300 displays a similar binding affinity to ondansetron [a current marketed therapeutic for nausea and vomiting (Zofran), used “off-label” to treat IBS-d] and ~ 10 times lower affinity than the very high-affinity 5-HT₃ receptor antagonist, alosetron (Hirata et al., 2007).

The potential therapeutic activity of CSTI-300 was evaluated in a rodent model devised to mimic primarily the increased visceral sensitivity of patients with IBS-d, which can be demonstrated by intraluminal bowel distension (Houghton et al., 2002), although this symptom is also relevant to patients with carcinoid syndrome (<https://www.cancer.org/cancer/gastrointestinal-carcinoid-tumor/detection-diagnosis-staging/signs-symptoms.html>). This rat in vivo model had been shown previously to respond to 5-HT₃ receptor antagonists predicting their efficacy (Banner and Sanger, 1995; Banner et al., 1995), which was subsequently established in patients with IBS-d and carcinoid syndrome. This preclinical model uses conscious rats in which a relevant noxious stimulus is delivered by the slow inflation of an intracolonic balloon; the pressure required to evoke an abdominal contraction being the quantitative readout (Banner and Sanger, 1995; Banner et al., 1995). Consistent with the previous studies (Banner and Sanger, 1995; Banner et al., 1995), peripheral administration of the precursor of 5-HT, 5-HTP, increased the sensitivity of

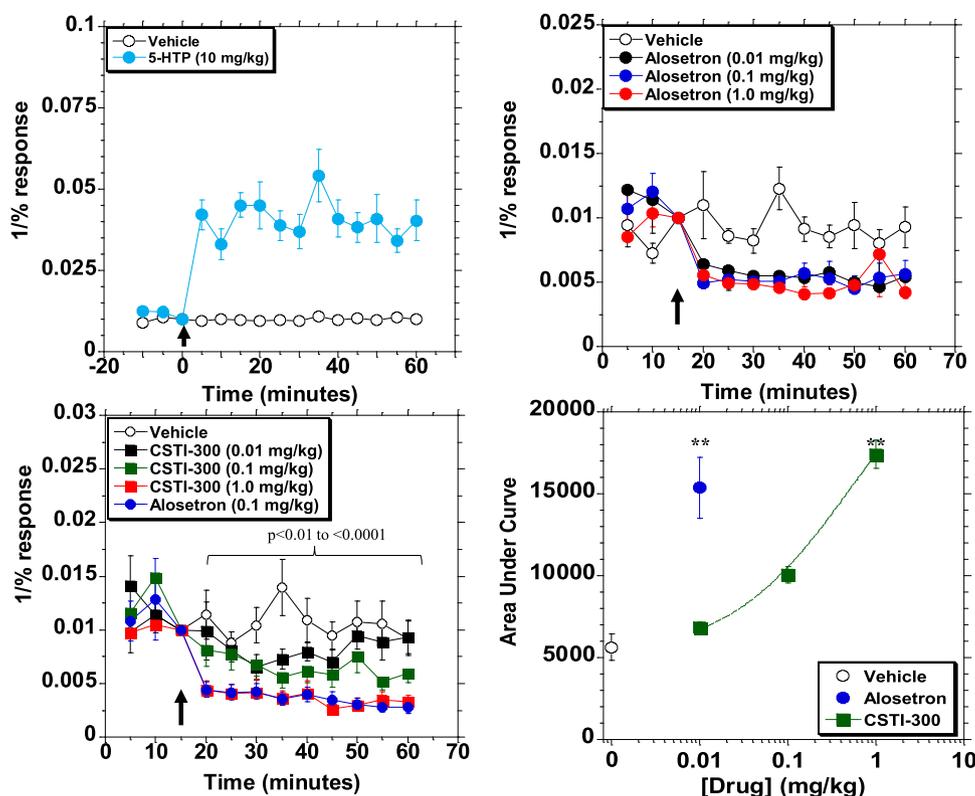


Fig. 7. Ability of CSTI-300 to display comparable efficacy to a maximal effective dose of alosetron in a rat model of IBS-d in vivo. Inflation of a balloon in the colon of conscious rats allowed colon sensitivity to be quantified by the balloon pressure required to evoke abdominal contraction. Top left: 5-HTP (10 mg/kg, s.c., arrow indicates time of administration), the precursor of 5-HT, increases the sensitivity of rats to colon distension by reducing the pressure required to evoke abdominal contraction. The impact of 5-HTP was statistically significant [$P < 0.0001$; two-way ANOVA with subsequent Tukey's multiple comparisons test (with Bonferroni correction for multiple comparisons)]. Top right: the 5-HTP-induced increase in colon sensitivity was reversed by the selective 5-HT₃ receptor antagonist, alosetron (see also bottom left panel for a further group of animals treated with alosetron; arrow indicates administration of vehicle or alosetron as appropriate). Bottom left: the 5-HTP-induced increase in colon sensitivity was reversed by CSTI-300 in a dose-dependent manner (arrow indicates administration of vehicle, CSTI-300 or alosetron as appropriate). The impact of alosetron or CSTI-300 was statistically significant [$P < 0.01$ to <0.0001 ; two-way ANOVA with subsequent Tukey's multiple comparisons test (with Bonferroni correction for multiple comparisons)]. Bottom right: dose-response curve for CSTI-300, generated by calculating area under the curve from the bottom left panel, yielded an ED₅₀ of 0.55 mg/kg. The impact of alosetron and CSTI-300 (1.0 mg/kg) was statistically significant ($P < 0.01$), determined by Kruskal-Wallis test with Dunn's multiple comparisons test. Data represent mean \pm S.E.M., $n = 10$.

rats to the colonic distension such that the abdominal contraction was evident at lower balloon pressures. This was likely because of the conversion of 5-HTP to 5-HT, which would increase the signaling tone upon the 5-HT₃ receptor; this also mimics the biochemical pathology reported in patients with IBS-d (Bearcroft et al., 1998; Dunlop et al., 2005; Enck et al., 2016; Fu et al., 2019; Gunn et al., 2019). This increased sensitivity to colon distension after 5-HTP administration was reversed by the clinically proven 5-HT₃ receptor antagonist, alosetron; this finding supported the validation of the model. Since CSTI-300 is also a partial agonist at the 5-HT_{3A} receptor, the ability of the lead candidate drug, CSTI-300, to display at least comparable activity to alosetron in this IBS-d model supports further development of this partial agonist as a treatment of patients with IBS-d and potentially

for relief of gastrointestinal symptoms associated with carcinoid syndrome. As part of this development, the ability of CSTI-300 to attenuate diarrhea in preclinical models should be evaluated to see whether the compound has potential benefit to relieve additional symptoms associated with IBS-d and carcinoid syndrome.

Although 5-HT₃ receptor antagonists from different chemical series display well-recognized efficacy in patients with IBS-d (e.g., alosetron, cilansetron, ondansetron, ramosetron; Andresen et al., 2008; Ford et al., 2009; Garsed et al., 2014; Fukudo et al., 2016) and relief from some symptoms for patients with carcinoid syndrome (Anderson et al., 1987; Jacobsen, 1992; Platt et al., 1992; Schworer et al., 1995; Wymenga et al., 1998; Kiesewetter and Raderer, 2013; Halperin et al., 2017), their propensity to induce sometimes

TABLE 1

CSTI-300 does not evoke emesis, alter the state of feces, or affect the general behavior of dogs after an oral dose of 1.0 mg/kg

The free plasma concentration taken 6 h after oral dosing was between 10 and 70 \times the K_d for the h5-HT_{3A} receptor (dog plasma protein binding 30% \pm 3%; unpublished observations). Data from six dogs. Impact of CSTI-300 on the behavior and gastrointestinal activity in dog.

Treatment	Emetic Episodes (Either Retching or Vomiting)	State of Feces	General Observations	Plasma [CSTI-300] 6 h Postdose Mean \pm S.D. (Range)
CSTI-300 (1.0 mg/kg p.o.)	No emetic episodes for any of the six dogs	Normal for all six dogs	No abnormal observations	105 \pm 87 nM (31–227 nM)

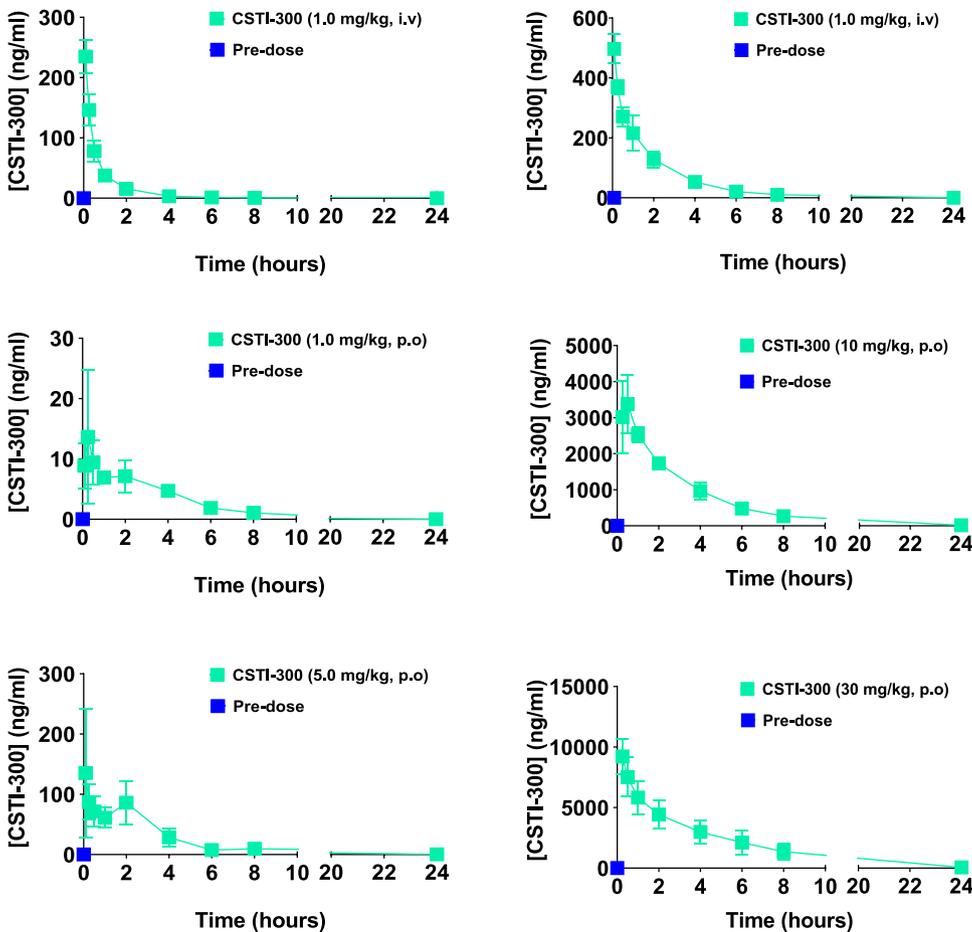


Fig. 8. The pharmacokinetics of CSTI-300 in rat and dog. In rat, after administration intravenously (1.0 mg/kg, top left) CSTI-300 had a half-life of 1.6 hours. With oral administration (1.0 mg/kg, middle left; 5.0 mg/kg, bottom left), CSTI-300 was metabolized with a half-life of approximately 2 hours for 1.0 mg/kg or 4 hours for 5.0 mg/kg. Data represent mean \pm S.D., $n = 3$. In dog, after administration intravenously (1.0 mg/kg, top right), CSTI-300 had a half-life of 1.6 hours. Regarding oral administration (10 mg/kg, middle right; 30 mg/kg, bottom right), CSTI-300 was metabolized with a half-life of approximately 4 hours. Data represent mean \pm S.D., $n = 4$.

serious side effects limits their utility. Thus, constipation can be a common side effect and can be severe in some cases (Chang et al., 2006; Andresen et al., 2008; Ford et al., 2009; Garsed et al., 2014; Fukudo et al., 2016; for systematic review see Zheng et al., 2017). In addition, rare instances of ischemic colitis have severely limited access of patients with IBS-d to these drugs. Although the pharmacological mechanism underlying the ischemic colitis is not understood, it likely arises from a combination of IBS-d and high levels of 5-HT₃ receptor antagonism (see *Introduction*).

The mechanism underlying constipation, however, is likely to be simply related to the level of 5-HT₃ receptor inhibition. Hence a 5-HT₃ receptor partial agonist such as CSTI-300 would be predicted to be less likely to cause constipation since some level of 5-HT₃ receptor activity will be retained, even at high dosage. Furthermore, the more modest, competitive binding affinity of CSTI-300 for the 5-HT₃ receptor relative to alosetron would allow the local extracellular 5-HT concentration to be more influential to promote receptor function.

TABLE 2

Pharmacokinetic profile of CSTI-300 in rat and dog

Data represented as mean \pm S.D. The pharmacokinetics of CSTI-300 in rat and dog.

Species (n)	Dose (mg/kg)	AUC _{last} ^a (h*ng/ml)	t _{max} ^b (h)	C _{max} ^c (ng/ml)	T _{1/2} ^d (h)	CL ^e (l/h per kilogram)	Vd ^f (l/kg)	%F ^g
Rat (3)	1.0, i.v.	163 \pm 27	0.083 \pm 0	235 \pm 28	1.6 \pm 0.2	6.2 \pm 1.0	14.6 \pm 4.3	
Rat (3)	1.0, p.o.	39.8 \pm 3.0	0.8 \pm 0.1	15 \pm 10	2.3 \pm 0.7			24
Rat (3)	5, p.o.	331 \pm 102	0.8 \pm 1.1	138 \pm 68	4.4 \pm 2.7			41
Dog (4)	1.0, i.v.	764 \pm 135	0.083 \pm 0	497 \pm 48	1.6 \pm 0.2	1.3 \pm 0.2	3.0 \pm 0.3	
Dog (3)	0.3, p.o.	205 \pm 105	0.5 \pm 0	86.1 \pm 34.4	1.6 \pm 0.18			89
Dog (3)	1.0, p.o.	1010 \pm 712	0.5 \pm 0	364 \pm 157	2.2 \pm 1.1			100
Dog (4)	10, p.o.	12,098 \pm 1076	0.4 \pm 0.1	3498 \pm 725	3.7 \pm 0.8			100
Dog (4)	30, p.o.	39,115 \pm 13,049	0.25 \pm 0	9223 \pm 1464	3.4 \pm 0.6			100

^aArea under the plasma concentration vs. time curve from 0 to the last time point CSTI-300 was quantifiable in plasma.

^bTime of maximum observed concentration of CSTI-300 in plasma.

^cMaximum observed concentration of compound in plasma.

^dApparent half-life of the terminal phase of elimination of CSTI-300 from plasma.

^eTotal body clearance of CSTI-300.

^fVolume of distribution.

^gOral bioavailability; F = (AUC_{last} p.o. \times Dose_{iv})/(AUC_{last} i.v. \times dose_{po}).

TABLE 3

Summary of additional pharmacological and pharmaceutical characteristics of CSTI-300
Further pharmaceutical characteristics of CSTI-300.

High affinity and selectivity for 5-HT ₃ receptor	No significant off-target activity when screened at 1.0 μM (see Supplemental Table 1 for list of targets)
Low probability of drug/drug interactions	CYPs (1A2, 2B6, 2C9, 2C19, 2D6, 3A4) IC ₅₀ > 100 μM
Good oral PK in rodent and dog	F _{mice} = 42%, t _{1/2} = 4.2 h F _{dog} = 100%, t _{1/2} = 3.7 h
Large margin of safety	NOAEL (no observed adverse effect level) = 300 mg/kg per day (7-day dosing in rat); 300 × ED ₉₀ dose Good tolerability in dog; no emesis or loose stool at 1 mg/kg (>10 × ED ₉₀ concentration) Nonmutagenic in micro-Ames Test
Excellent pharmaceutical/biophysical properties	MM = 296.37 Da Log P = 1.95 Water soluble (>400 mg/ml)
Plasma protein binding	Human: 7% ± 4% Rat: 17% ± 5% Dog: 30% ± 3%

ED₉₀, 90% effective dose; MM, molecular mass; NOAEL, no observed adverse effect level; PK, pharmacokinetics.

Interestingly, there is a previous example of a 5-HT₃ receptor partial agonist being evaluated in patients with IBS, but it occurs in IBS-c rather than IBS-d. The rationale was that the intrinsic activity of pumosetrag (DDP733; MKC-733) for the 5-HT₃ receptor would promote motility within the gastrointestinal tract to reverse the symptoms, as had been predicted from rodent studies (Chetty et al., 2008). In human trials, although pumosetrag increased small intestinal transit and improved bowel habits of patients with IBS-c (Coleman et al., 2003; Fujita et al., 2005), mild nausea and abdominal discomfort were also reported, along with flushing (Evangelista, 2007), consistent with too much 5-HT₃ receptor activation by the drug. Nausea has also been associated with administration of another 5-HT₃ receptor agonist (Staner et al., 2001), and hence a potential concern would be that CSTI-300 may manifest similar emetic episodes in patients that would limit the potential clinical utility of the drug. In the present study, the potential of CSTI-300 to evoke emesis (along with monitoring for other potential adverse events) was directly assessed by oral administration of CSTI-300 to dogs at a dose resulting in relatively high plasma drug concentrations predicted to saturate the 5-HT₃ receptor. In these studies, CSTI-300 failed to induce vomiting or to change the behavior of the dog experiencing nausea. Furthermore, there was no evidence of increased gastric motility in these animals. It is acknowledged that no positive-control emetogen was included in these studies (e.g., cisplatin) although it is generally considered that dogs are relatively sensitive to emetogens, hence the inability of CSTI-300 to induce emesis or other abnormal behaviors in dogs is considered noteworthy by the authors. Additional studies of CSTI-300 in dogs and mini-pigs conducted primarily to further assess the pharmacokinetics of the drug, failed to evoke emetic episodes at doses of CSTI-300 up to 30 mg/kg p.o. (Guzzo et al., unpublished observations). Such findings support that CSTI-300 may be able to deliver efficacy to patients with IBS-d and carcinoid syndrome without side effects of nausea/vomiting or diarrhea from too much stimulation of the 5-HT₃ receptor. Indeed, Phase I clinical studies with CSTI-300 are now planned that will include monitoring of potential to modify gastrointestinal motility as well as the potential to evoke emesis.

Of relevance to the present studies, vortioxetine, a multimodal, well-tolerated, and efficacious antidepressant (Cipriani et al., 2018) displays 5-HT₃ receptor partial agonist actions

(Bang-Andersen et al., 2011; Ladefoged et al., 2018), and a case report describes the symptomatic relief of a patient with IBS-d by this drug (Aydin and Ünal-Aydin, 2017). This further strengthens the argument that CSTI-300 would make a good therapeutic for IBS-d.

It is intriguing to speculate that the mixed functional action of CSTI-300 at the 5-HT₃ receptor dependent on the prevailing local 5-HT concentration may offer further therapeutic benefit. Thus, although the ability of CSTI-300 to reduce 5-HT₃ receptor function in the presence of elevated 5-HT levels may offer clinical efficacy to patients with IBS-d, a degree of 5-HT₃ receptor activation may benefit those with low 5-HT levels experiencing constipation (Dunlop et al., 2005; Atkinson et al., 2006). It may therefore transpire that CSTI-300 may find utility to treat IBS-m, in which the symptoms fluctuate between diarrhea and constipation and for which there are no current licensed medications.

In addition to a potential treatment of patients with IBS, CSTI-300 would be predicted to be beneficial to control the sometimes debilitating gastrointestinal discomfort of patients with carcinoid syndrome, often an indolent metastatic neuroendocrine tumor that secretes copious quantities of 5-HT with patients suffering for many years (Davis et al., 1973; Kvols, 1994; Halperin et al., 2017). The gastrointestinal symptoms may be reduced by the somatostatin analogs, octreotide or lanreotide, but often not sufficiently (e.g., Kvols et al., 1986; Fisher et al., 2018). In March 2017, telotristat, a novel inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis, was approved by the FDA for the management of carcinoid syndrome symptoms refractory to somatostatin analogs, although the predictable side effects of constipation and depression were evident along with deranged liver function tests (Chan and Singh, 2018; Pavel et al., 2018), indicating a continued need for safe efficacious drugs for this indication. Notably, previous studies have shown patients with carcinoid syndrome display dramatic and immediate clinical benefit after administration of 5-HT₃ receptor antagonists (Anderson et al., 1987; Jacobsen, 1992; Platt et al., 1992; Schworer et al., 1995; Wymenga et al., 1998; Kiesewetter and Raderer, 2013), and in the presence of the considerably elevated 5-HT levels, partial agonists like CSTI-300 would be predicted to display efficacy with a reduced risk of constipation that can also be experienced when 5-HT₃ receptor antagonists

are used for this indication (Kiesewetter and Raderer, 2013).

In summary, CSTI-300 is a high-affinity 5-HT₃ receptor partial agonist that displays efficacy in a rodent model of IBS-d with some relevance to carcinoid syndrome. The drug displays good oral pharmacokinetics as well as desirable pharmaceutical characteristics, making it a promising candidate for subsequent clinical trial to evaluate the potential to deliver symptomatic relief to patients with for example IBS-d and carcinoid syndrome.

Authorship Contributions

Participated in research design: Manning, Moore, Sargent, Guzzo, Barnes.

Conducted experiments: Roberts, Grafton, Powell, Chen, Xie, Huang, Liu, Brady, Qureshi, Stamataki, Manning, Moore, Sargent, Guzzo, Barnes.

Performed data analysis: Brock, Cooper, Manning, Moore, Sargent, Guzzo, Barnes.

Wrote or contributed to the writing of the manuscript: Barnes with input from all authors.

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CSTI-300; a novel 5-HT₃ receptor partial agonist with potential to treat patients with irritable bowel syndrome or carcinoid syndrome

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Supplementary Table 1

Target	Company	Species
5-HT _{1A} receptor	Cerep and MDS Pharma Services	Human
5-HT _{1B} receptor	Cerep	Rat
5-HT _{1D} receptor	Cerep	Rat
5-HT _{2A} receptor	Cerep	Human
5-HT _{2B} receptor	Cerep	Human
5-HT _{2C} receptor	Cerep	Human
5-HT _{4e} receptor	Cerep	Human
5-HT _{5A} receptor	Cerep	Human
5-HT ₆ receptor	Cerep	Human
5-HT ₇ receptor	Cerep	Human
Adenosine A ₁ receptor	MDS Pharma Services	Human
Adenosine A _{2A} receptor	MDS Pharma Services	Human
Adenosine A ₃ receptor	MDS Pharma Services	Human
Adrenergic α_{1A} receptor	MDS Pharma Services	Rat
Adrenergic α_{1B} receptor	MDS Pharma Services	Rat
Adrenergic α_{1D} receptor	MDS Pharma Services	Human
Adrenergic α_{2A} receptor	MDS Pharma Services	Human
Adrenergic β_1 receptor	MDS Pharma Services	Human
Adrenergic β_2 receptor	MDS Pharma Services	Human
Androgen (Testosterone) receptor	MDS Pharma Services	Rat
BK (K _{Ca} 1.1)	ChanTest	Human
Bradykinin B ₁ receptor	MDS Pharma Services	Human

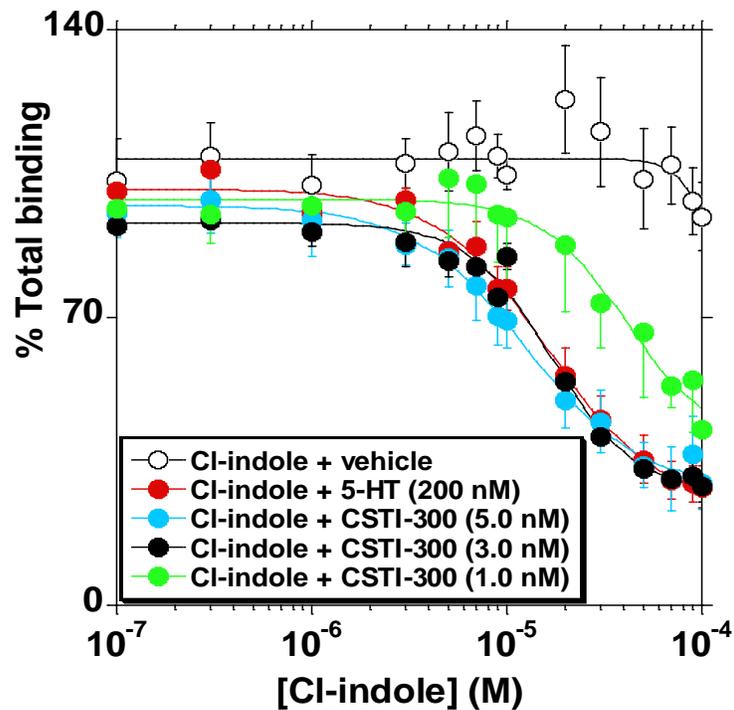
Bradykinin B ₂ receptor	MDS Pharma Services	Human
Calcium Channel L-Type, Benzothiazepine	MDS Pharma Services	Rat
Calcium Channel L-Type, Dihydropyridine	MDS Pharma Services	Rat
Calcium Channel L-Type (Cav1.2)	ChanTest	Human
Calcium Channel N-Type	MDS Pharma Services	Rat
Calcium Channel T-type (Cav3.2)	ChanTest	Human
Chloride Channel-1	ChanTest	Human
Cysteinyl Leukotriene CysLT ₁ receptor	MDS Pharma Services	Human
Cystic Fibrosis Transmembrane Conductance Regulator	ChanTest	Human
Dopamine D ₁ receptor	MDS Pharma Services	Human
Dopamine D _{2S} receptor	MDS Pharma Services	Human
Dopamine D ₃ receptor	MDS Pharma Services	Human
Dopamine D _{4,2} receptor	MDS Pharma Services	Human
Dopamine Transporter	MDS Pharma Services	Human
Endothelin ET _A receptor	MDS Pharma Services	Human
Endothelin ET _B receptor	MDS Pharma Services	Human
Epidermal Growth Factor	MDS Pharma Services	Human
GABA Transporter	MDS Pharma Services	Rat
GABA _A , Flunitrazepam receptor	MDS Pharma Services	Rat
GABA _A , Muscimol receptor	MDS Pharma Services	Rat
GABA _{B1A} receptor	MDS Pharma Services	Human
Glucocorticoid receptor	MDS Pharma Services	Human
GPR103 receptor	MDS Pharma Services	Human
HCN2	ChanTest	Human

HCN4	ChanTest	Human
hERG	Chantest and MDS Pharma Services	Human
Histamine H ₁ receptor	MDS Pharma Services	Human
Histamine H ₂ receptor	MDS Pharma Services	Human
Histamine H ₃ receptor	MDS Pharma Services	Human
IK (K _{Ca} 3.1)	ChanTest	Human
Imidazoline I ₂ receptor	MDS Pharma Services	Rat
Interleukin IL-1 receptor	MDS Pharma Services	Mouse
Kainate receptor	MDS Pharma Services	Rat
K _{ATP}	MDS Pharma Services	Hamster
Kir2.1	ChanTest	Human
Kir3.1/3.4	ChanTest	Human
Kir6.2/SUR2A	ChanTest	Human
Kv1.3	ChanTest	Human
Kv1.5	ChanTest	Human
Kv4.3	ChanTest	Human
KvLQT/minK	ChanTest	Human
Melatonin MT ₁ receptor	MDS Pharma Services	Human
Muscarinic M ₁ receptor	MDS Pharma Services	Human
Muscarinic M ₂ receptor	MDS Pharma Services	Human
Muscarinic M ₃ receptor	MDS Pharma Services	Human
Nicotinic α -BGTX-insensitive ($\alpha_4\beta_2$) receptor	Cerep	Rat
Nicotinic α -BGTX-sensitive (α_7) receptor	Cerep	Rat
Nav1.5 Phasic	ChanTest	Human

Nav1.5 Tonic	ChanTest	Human
Neuropeptide Y Y ₁ receptor	MDS Pharma Services	Human
Neuropeptide Y Y ₂ receptor	MDS Pharma Services	Human
Nicotinic Acetylcholine receptor	MDS Pharma Services	Human
Nicotinic Acetylcholine α_1 Bungarotoxin receptor	MDS Pharma Services	Human
NMDA, Agonism receptor	MDS Pharma Services	Rat
NMDA, Glycine receptor	MDS Pharma Services	Rat
NMDA, Phencyclidine receptor	MDS Pharma Services	Rat
Noradrenaline Transporter	MDS Pharma Services	Human
Oestrogen ER α receptor	MDS Pharma Services	Human
Opiate δ receptor	MDS Pharma Services	Human
Opiate κ receptor	MDS Pharma Services	Human
Opiate μ receptor	MDS Pharma Services	Human
P2X ₁ receptor	ChanTest	Human
P2X ₃ receptor	ChanTest	Human
Phorbol Ester	MDS Pharma Services	Mouse
Platelet Activating Factor	MDS Pharma Services	Human
Prostanoid EP ₄ receptor	MDS Pharma Services	Human
Purinergic P2X receptor	MDS Pharma Services	Rabbit
Purinergic P2Y receptor	MDS Pharma Services	Rat
Rolipram	MDS Pharma Services	Rat
Serotonin Transporter	MDS Pharma Services	Human
Sigma σ_1 receptor	MDS Pharma Services	Human
Sigma σ_2 receptor	MDS Pharma Services	Rat
Sodium Channel Site 2	MDS Pharma Services	Rat

Tachykinin NK ₁ receptor	MDS Pharma Services	Human
Thyroid Hormone	MDS Pharma Services	Rat
TRPV1	ChanTest	Human

Supplementary Table 1: Pharmacological selectivity of CSTI-300. CSTI-300 (1.0 μ M) failed to interact with a wide variety of named targets assessed by the screening assays performed by either Cerep (www.cerep.fr), ChanTest (www.criver.com) or MDS Pharma Services (www.mdsp.com). For all the pharmacological targets named above, CSTI-300 displayed affinity \ll 1 μ M.



Competing ligand	IC ₅₀ (μM)
Cl-indole + vehicle	>> 100 μM
Cl-indole + 5-HT (200 nM)	25±6*
Cl-indole + CSTI-300 (1.0 nM)	63±14
Cl-indole + CSTI-300 (3.0 nM)	30±4*
Cl-indole + CSTI-300 (5.0 nM)	24±5**

Supplementary Figure 1. Consistent with agonist activity, CSTI-300 reveals the ability of Cl-indole to compete at the h5-HT_{3A} receptor. The ability of CSTI-300 (or 5-HT) to modify the ability of Cl-indole to compete for [³H]-granisetron binding to the h5-HT_{3A} receptor. Data

represents mean \pm SEM, n=5. Table insert; apparent affinity of Cl-indole. *p<0.05,
**p<0.01(Kruskal-Wallis test with Dunn's multiple comparisons test).