JNJ-26070109 [(R) 4-Bromo-N-[1-(2, 4-difluoro-phenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide]: A novel, potent and selective cholecystokinin 2 receptor antagonist with good oral bioavailability.

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JNJ-26070109: A novel CCK2 receptor antagonist

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Abbreviations: CCK, Cholecystokinin; E/[A], concentration-effect; GPCR, guanine nucleotide coupled receptor; JNJ-26070109, [(R) 4-Bromo-N-[1-(2,4-difluorophenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide]; CHO, Chinese hamster ovary, HEKZFP, human embryonic kidney zinc finger protein; BH-CCK-8S, Bolton Hunter-sulfated cholecystokinin octapeptide; YF476, (R)-1-[2,3-dihydro-2-oxo-1-pivaloylmethyl-5-(2'-pyridyl)-1H-1,4-benzodiazepin-3-yl]-3-(3-methyl-phenyl)urea.

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Abstract
JNJ-26070109 [(R) 4-Bromo-N-[1-(2,4-difluoro-phenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide] is a representative of a new chemical class of competitive antagonists of CCK2 receptors. In this study, the primary in-vitro pharmacology of JNJ-26070109 was evaluated along with the pharmacokinetic and pharmacodynamic properties of this compound in rat and canine models of gastric acid secretion. JNJ-26070109 expressed high affinity for human (pK_I = 8.49±0.13), rat (pK_I = 7.99±0.08) and dog (pK_I = 7.70±0.14) CCK2 receptors. The selectivity of JNJ-26070109 at the CCK2 receptor versus the CCK1 receptor was species-dependent, with the greatest degree of selectivity (>1200-fold) measured at the human isoforms of the CCK receptors (selectivity at CCK2 versus CCK1 receptors: human ~1222-fold, rat ~324-fold, dog ~336-fold). JNJ-26070109 behaved as a surmountable, competitive, antagonist of human CCK2 receptors in a calcium mobilization assay (pK_B = 8.53±0.05) and in pentagastrin-stimulated gastric acid secretion in the isolated, lumen-perfused, mouse stomach assay (pK_B = 8.19±0.13). The pharmacokinetic profile of this compound was determined in vivo in rats and dogs. JNJ-26070109 was shown to have high oral bioavailability (%F rat = 73±16, %F dog = 92±12) with half lives of 1.8±0.3 h and 1.2±0.1 h in rat and dog, respectively. The pharmacodynamic properties of this compound were investigated using two in-vivo models. In conscious rat and dog chronic gastric fistula models of pentagastrin-stimulated acid secretion, JNJ-26070109 had oral EC_{50} values of 1.5 μM and 0.26 μM, respectively. Overall, we have demonstrated that JNJ-26070109 is a high
affinity, selective, CCK2 receptor antagonist with good pharmacokinetic properties.
Introduction

Gastrin, acting through the CCK2 receptors, regulates a number of biological functions including gastric acid secretion, anxiety, satiety and growth of the gastrointestinal mucosa (see Noble et al., 1999, Miyake et al., 1994; Wettstein et al., 1994; Baldwin, 1995). CCK2 receptors have also been implicated in the pathophysiology of cancer (Takhar et al., 2004; Baldwin, 1995) and Barrett’s esophagus (Haigh et al., 2003). The dual function of CCK2 receptors in regulating gastric acid secretion and growth of the gastrointestinal mucosa make this an attractive and novel target for the treatment of gastroesophageal reflux disease. Thus, potentially, antagonism of these receptors could reduce the total acid secretory capacity of the stomach through growth modulatory effects in combination with the inhibition of acid secretion. Alternatively, CCK2 receptor antagonists could provide an adjunct therapy to proton pump inhibitors (PPIs) with the aim of reducing the effects of PPI-associated hypergastrinemia (Scarpignato et al., 2006). Taken together, it is possible that CCK2 antagonists would offer advantages over the current standard of care for GERD (histamine H2 receptor antagonists or proton pump inhibitors) which have clinical limitations including delayed time to onset, nocturnal acid breakthrough and rebound hypersecretion following cessation of treatment (see Parsons & Keeling, 2005; Gillen et al., 1999; Reimer et al., 2009).
There are several distinct chemical classes of CCK2 receptor antagonists which have previously been identified through drug discovery programs. These small molecules include benzodiazepine-based compounds (L-365,260, YM022 and YF476), peptoids (PD-134,308) and indoles (JB93182; for review see Herranz, 2003). In addition, a vaccine aimed to neutralize gastrin (Gastrimmune or INSEGIA\textsuperscript{TM}; Aphton Corporation) has also been developed and this therapy showed promise in Phase II clinical trials for gastric, colorectal and pancreatic carcinoma (Gilliam and Watson, 2007). However, despite the variety of CCK2 receptor-targeted ligands, no compounds are currently available for clinical use. This may be because many compounds in this class have been associated with poor or variable pharmacokinetics, both with respect to gastrointestinal absorption as well as blood-brain barrier penetration (Herranz, 2003). For example, the CCK2 receptor antagonist, JB95008, demonstrated efficacy in Phase II trials of pancreatic cancer, however, this compound had to be administered by continuous intravenous infusion due to its poor oral bioavailability (Chau et al., 2006). In addition, some CCK2 receptor antagonists express limited selectivity between the CCK2 and CCK1 receptors (e.g. L-365,260 is \textasciitilde25-fold selective and YF476 is \textasciitilde100-fold selective, data taken from Morton et al., 2003 and Morton et al., 2005) which may, depending on the clinically efficacious dose, prohibit selective antagonism of the CCK2 receptors in patients. We initiated a program aimed at discovering small molecule CCK2 receptor antagonists driven by structural novelty, developable biopharmaceutical properties and good pharmacokinetic properties.
The novel CCK2 receptor antagonist described here, JNJ-26070109, is the best-characterized compound resulting from medicinal chemistry optimization around a lead molecule identified by high-throughput screening of the J&JPRD compound collection (Allison et al., 2006; Woods et al., 2007; Rosen et al., 2008). Where feasible, the CCK2 receptor antagonist, YF476 was included as an internal control in the assay. JNJ-26070109 expressed high affinity and good selectivity for human, mouse, rat and canine CCK2 receptors and behaved as a surmountable competitive antagonist in in vitro and acute, in vivo bioassays of acid secretion. Furthermore, JNJ-26070109 had good oral bioavailability as evidenced by its pharmacokinetic profile in rats and dogs and in vitro human ADME assays. Overall, JNJ-26070109 is a novel CCK2 receptor antagonist which was considered suitable for clinical investigation.
Methods

All procedures and experiments were performed according to the internationally accepted guidelines for the care and use of laboratory animals in research, and they were approved by the local Institutional Animal Care and Use Committee or the Beerse Institutional Ethical committee.

Cell culture

Chinese hamster ovary-K cells that had undergone stable transfection with the human, rat or canine CCK1 receptor or the rat and canine CCK2 receptor were maintained in Dulbecco’s modified Eagles’s medium (Ham’s F-12) supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 50 U ml⁻¹ penicillin, 50 μg ml⁻¹ streptomycin and 0.6 mg ml⁻¹ Geneticin for continuous selection (all materials from Invitrogen, Carlsbad, CA). For radioligand binding studies, the cells were harvested by cell scraping, and resulting pellets were immediately frozen at -80°C (approximately 50 x 10⁶ cells/pellet).

Radioligand binding studies

Frozen pellets of CHO cells, stably transfected with the CCK1 or CCK2 receptor of interest, were used. For the human CCK2 receptor assay, HEK cells which had been transfected with selective zinc finger proteins to up-regulate human CCK2 receptor expression were used (HEKZFP system; for details see Morton et al., 2005). All cell pellets were defrosted on ice in 15 ml of assay buffer (10 mM HEPES, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl₂, and 0.089 mM bacitracin, pH
7.2 at 21±3°C) and then they were homogenized in a Polytron homogenizer (setting 10, 7 x 3 s; Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged (800g for 5 min at 4°C) and the pellet discarded. The supernatant was recentrifuged (26,892g for 25 min at 4°C) and the final pellet was resuspended in assay buffer. Protein concentration was determined using BCA Protein Assay Kit (Pierce Chemical, Rockford, IL). All binding assays were conducted in 96-well Multiscreen GF/B filter plates (Millipore Corporation, Billericay, MA) that were pre-soaked in assay buffer for 1 h. For competition studies, cell membranes (45 μl) were incubated with 60 pM [125I]-BH-CCK-8S (50 μl) in the presence of competing ligand (15 μl) for 100 min (total volume of 150 μl). Nonspecific binding was determined by inclusion of 1 μM JB93182 (a highly selective CCK2 receptor antagonist; Harper et al., 1996). The bound radioactivity was separated by filtration using a Multiscreen Resist manifold (Millipore Corporation). The filters were washed three times with ice-cold PBS (pH 7.5) and radioactivity retained on the filters was measured by liquid scintillation counting using a TopCount (PerkinElmer Life and Analytical Sciences Boston, MA). Radioligand binding studies on human gallbladder membranes were conducted as previously described (Morton et al., 2002) using [3H]-L-364,718 as radiolabel.

**Calcium mobilization measured using FLIPR**

Cells were seeded into black-walled 384well plates (Biocoat BD Biosciences) at 5,000 cells/well and grown for 24 h in culture medium before stimulating the
receptor expression with 2 ng/ml of Doxycyclin for 48 h. On the day of the experiment, cells were pre-incubated with Devazepide at 100 nM for 20 min prior to the addition of different concentrations of JNJ26070109 and calcium assay kit solution (BD Biosciences) containing 50 µM probenecid (Sigma-Aldrich) for 45 min at 37°C and 15 min at room temperature. The intracellular Ca^{2+} flux was assayed using a FLIPR Tetra (Molecular Devices) to simultaneously monitor fluorescence in all wells (λ excitation = 488 nm, λ emission = 540 nm). Cells were challenged with different concentrations of CCK8-S (Sigma-Aldrich) agonist peptide (delivered at a velocity of 20 ml s⁻¹) and the fluorescence intensity was captured every 1 s for 1 min then every 6 s for 3 min after agonist addition.

**Receptor binding at a panel of additional pharmacological targets for selectivity determination**

The selectivity of JNJ-26070109 was assessed in a standard panel of radioligand-binding assays for 50 additional pharmacological loci (standard selectivity screening panel from CEREP, Celle L'Evescault, France. JNJ-26070109 was tested at 1 µM. All studies were internally controlled with reference ligands.

**In vitro, lumen-perfused, mouse stomach acid secretion assay**

Gastric acid secretion was measured in the mouse, isolated, lumen-perfused, stomach preparation (Black & Shankley, 1985). Isolated whole stomachs were removed from fasted, young adult mice (22-26 g) of either gender, following
euthanasia by asphyxiation using a rising concentration of CO₂. The abdomen was opened and the stomach cannulated via the duodenal sphincter. The oesophagus was ligated at the level of the cardiac sphincter and the stomach excised from the abdomen. A small incision was made in the fundic region, a cannula ligated tightly into the incision and the contents of the stomach flushed through with mucosal solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂, 31.6 mM glucose) to remove any remaining food. The stomach was placed into an organ bath containing 30 ml of buffered serosal solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.14 mM KH₂PO₄, 15.9 mM Na₂HPO₄, 0.065 mM CaCl₂, 31.6 mM glucose). The serosal solution was maintained at 37±1°C and gassed vigorously with 95% O₂ and 5% CO₂. The stomachs were perfused with unbuffered mucosal solution gassed with 100% O₂ at a rate of 1 ml min⁻¹ and the perfusate passed over a pH electrode system. The pH of the effluent was recorded as an index of gastric acid secretion (data recorded using PowerLab (ADI instruments, CO, USA). The preparations were allowed to stabilize for 60 min before the addition of drugs which were added directly to the serosal solution in the organ bath. Antagonists were allowed to equilibrate for 60 min prior to obtaining a single, cumulative, pentagastrin concentration-effect curve by dosing at log unit intervals (0.1 nM to 10 μM). Pentagastrin-stimulated responses were expressed as the change in pH.

Inhibition of CCK-8S induced guinea-pig gallbladder contraction using JNJ-26070109
This method was conducted as described previously (Morton et al., 2007). Male Hartley guinea pigs (300-650 g) were euthanized using a rising concentration of CO₂. A midline incision was made into the abdominal cavity, the gallbladder removed and cleared of any adherent liver and connective tissue. Tissue strips were dissected from the central band of the gallbladder in a circular orientation (1x4 mm; mucosa intact) and mounted in 20 ml organ baths containing Krebs-Henseleit solution (118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.0 mM Na₂HPO₄, 25 mM NaHCO₃ and 10 mM D-glucose; Sigma Chemical, Poole, Dorset, U.K.) at 29°C and continuously gassed with O₂, CO₂ (95:5). A resting tension of 1 g was applied to the tissue strips, and they were then equilibrated for 1 h during which time the buffer was replaced at 30-min intervals. CCK-8S concentration-effect (E/[A]) curves were obtained by cumulative dosing in each tissue, and once a maximal response was obtained, the preparations were washed by replacing the buffer at 10 min intervals until the response returned to baseline. Antagonists, were equilibrated for 90 min before another CCK-8S concentration-effect curve was obtained (repeat-curve design). Contractile, tissue responses were measured using isometric transducers and displayed on dual channel flat-bed recorders (Kipp and Zonen, Deift, The Netherlands).

In vitro pharmacokinetic analysis of JNJ-26070109 using Caco-2 cells

The absorption potential of JNJ-26070109 was evaluated using the human intestinal cell line Caco-2 (experiments conducted by Absorption Systems, Exton,
PA). In summary, Caco-2 monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. The permeability assay buffer was Hank’s Balanced Salt Solution containing 10 mM HEPES and 15 mM glucose at a pH of 7.0. JNJ-26070109 was added to the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37°C with 5% CO₂ and 90% relative humidity. At 1 and 2 h following addition, a 200 μl aliquot was taken from the receiver chamber and replaced with fresh assay buffer. Permeability through a cell-free (blank) membrane was studied to determine non-specific binding and free diffusion of the compound through the device. The flux of the dye, Lucifer yellow, was also measured for each monolayer after being subjected to the test compounds to ensure no damage was inflicted to the cell monolayers during the incubation period. All samples were assayed by high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 x 50 mm, 3 or 5 μM particle size, Phenomenex, Torrance, CA).

**In-vitro pharmacokinetic profiling of JNJ-26070109 in human hepatocytes**

Pharmacokinetic analysis of JNJ-26070109 in human hepatocytes was conducted by Absorption Systems, Exton, PA. In summary, viable hepatocytes from three donors (two female and one male) were purchased from Xenotech L.L.C. (Kansas City, KS). These were separated by centrifugation in a Percoll gradient and pooled at 1.5 x 10⁶ cells/ml in Krebs-Henseleit Buffer. The
experiments were initiated by the addition of 1 mM of test compound stock solution in 100% DMSO to 1.2 ml of cell suspension. The final test compound concentration was 5 μM. The cell suspension was immediately divided into three aliquots and incubated in a shaking water bath at 37°C. At 15, 30, 60 and 120 min, 60 μl of cell suspension were removed and mixed with 180 ml of 100% acetonitrile. The samples were then vortexed and sonicated for 5 min. After centrifugation, at 148g for 15 min, the supernatant was diluted with 2 volumes of distilled H2O and analyzed by high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 x 50 mm, 3 or 5 μM particle size, Phenomenex, Torrance, CA). Testosterone (20 μM) was included as an internal reference. Data are reported as the percent remaining of triplicate samples.

Cross-species plasma protein binding was conducted by Absorption Systems (Exton, PA) using an ultracentrifugation based method and following their standard protocols.

**In-vivo pharmacokinetics of JNJ-26070109**

The pharmacokinetic profile of JNJ-26070109 was assessed in male Sprague-Dawley rats (230-350 g) and in male beagle dogs (8-14 kg). In all studies, the intravenous (i.v.) formulation was prepared in 5% (v/v) N-methyl-2-pyrrolidone in 20% (w/v) HP-beta-CD (cyclodextrin) solution. For the oral formulation, JNJ-26070109 was mixed with 1 molar equivalent of arginine, followed by the addition
of a solution consisting of 10% water in PEG 400. The resulting mixture was stirred at room temperature until a solution was obtained (overnight). For the rat studies, 2 µmol kg\(^{-1}\) of JNJ-26070109 was administered by oral gavage to animals that had been fasted for 18 h. An additional group of rats were given 2 µmol kg\(^{-1}\) JNJ-26070109 via the tail vein. All rats were allowed water \textit{ad libitum} and those fasted were re-fed 4 h following oral dosing. For the canine studies, 18.3 µmol kg\(^{-1}\) was administered orally and 5.5 µmol kg\(^{-1}\) was bolus dosed via brachial vein puncture. Blood samples were collected from the tail vein in rats (250 µl) or from the saphenous vein in dogs (4-5 ml) at various time intervals (up to 26 h) and stored on melting ice for approximately 30 min prior to centrifugation (10 min at 3000 g). Plasma was collected and frozen at -20°C pending analysis of JNJ-26070109 using high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 x 50 mm, 3 or 5 µM particle size, Phenomenex, Torrance, CA). The lower limit of quantification (LLoQ) was 0.03 µM in both rat and dog plasma.

\textbf{Inhibition of pentagastrin-stimulated gastric acid secretion in anesthetized rats: the Ghosh and Schild model}

Male Sprague-Dawley rats (230-350 g) were anesthetized with isoflurane (1-3 %) mixed in room air. Gastric acid secretion was measured using the Ghosh & Schild preparation (Ghosh & Schild, 1958) with slight modifications. In brief, the carotid artery and jugular veins were cannulated to allow measurement of blood
pressure and for infusion of pentagastrin, respectively. The femoral vein was also cannulated and used for bolus administration of test compounds. The stomach was cannulated with an inflow tube positioned near the greater curvature of the stomach and an outflow cannula inserted into the duodenum. The esophagus was ligated to prevent back flow of the mucosal solution. The stomach lumen was washed with 20-60 ml of a non-buffering solution (mucosal solution: 135 NaCl mM, 4.8 mM KCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂ and 31.6 mM glucose maintained at 37°C and gassed with 95% O₂). The mucosal solution was continuously perfused through the stomach at the rate of 1 ml min⁻¹ using a Carter multichannel precision pump (Manostat, IL, USA). The perfusate was then passed over a pH electrode system (Beckman Instrument Inc., CA, USA) to allow the pH of the effluent from the stomach to be monitored continuously using a digital data acquisition system (ADI Instruments, CO, USA). Preliminary agonist dose-response studies demonstrated that infusion of 100 nmol kg⁻¹h⁻¹ pentagastrin produced ~80% of the maximal response (ED₈₀) and this concentration was continuously infused for subsequent antagonist studies. To determine the effects of JNJ-26070109, the compound was administered according to a cumulative dosing regime (10 nmol kg⁻¹ to 10 μmol kg⁻¹ when a stable response was obtained to the pentagastrin infusion after ~30 min). Following each dose of antagonist, blood samples were taken in order to determine the corresponding plasma levels. Changes in the pH of the stomach perfusate were monitored continuously.
Inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats: the gastric fistula model

Male, Sprague Dawley, rats (160-190 g), fasted for 18 h (water ad libitum), were anesthetized with isoflurane (1-3%) mixed in clean dry air and fitted with a stainless steel gastric fistula. Analgesia was provided for three days post-operatively (0.03 mg g⁻¹ buprenorphine subcutaneously, every 12 h). A post-operative recovery period of 7-10 days was allowed before experimentation. During this recovery period the rats were conditioned by having their stomachs rinsed with unbuffered gastric mucosal physiological solution (135 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂ and 31.6 mM glucose at pH 6.6) and by being placed for 3 h in the metabolic cages used to house the animals during gastric acid secretion collection. The rats adapted readily and rapidly to the fistula, and operated animals were maintained for as long as 4 months without complications.

Test compounds were administered orally 45 min before the stomach was flushed with 50-60 ml of warm mucosal solution to remove any residual gastric contents. A tube was then inserted in the fistula and the gastric secretions were collected under gravity into a volumetric cylinder. For these studies, animals were housed in modified metabolic cages and were unrestrained for the duration of the experiment. A single subcutaneous injection of 5 ml of normal saline was given to offset dehydration due to collection of the gastric secretion. Basal acid
secretion was determined over a 90 min period and secretagogue-stimulated secretion was assessed for a further collection period of 90 min following subcutaneous administration of 30 nmol kg$^{-1}$ pentagastrin. Aliquots were collected every 30 min and the amount of acid secreted was determined by titration with 0.01 N NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau, Switzerland). The values were pooled for the basal and secretagogue-stimulated periods (90 min each) and were expressed as total acid secretion (H$^+$ moles). Blood samples were taken at the end of the experiment (~4 h after dosing) for determination of the plasma concentration of JNJ-26070109. A washout period of 2-4 days was allowed between experiments on the same animal.

**Measurement of gastric acid secretion in conscious gastric-fistula dogs**

Female beagles (9-12 kg) instrumented with chronic gastric and duodenal fistulae were used for these studies. Experiments were performed using six dogs, allocated to treatment or vehicle, fasted for up to 18 h prior to experimentation. Dogs were positioned in Pavlov-frames and following washout of residual stomach content, gastric secretions were collected by gravity drainage from the gastric fistula for four 15 min periods. The volume of secretion was measured and total acid secretion determined by titration of a 0.25 ml sample with 0.01 N NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau, Switzerland).
Following a 45 min basal acid collection period, doses of JNJ-26070109 (0.55, 1.8, 5.5 μmol/kg) were administered intraduodenally via the duodenal fistula (12.5 ml). After a further 45 min, during which time the fistula was closed, a sub-maximal dose of pentagastrin was administered subcutaneously (4 μg kg\(^{-1}\)). Gastric secretions were collected for a further 90 min in 15 min aliquots and the amount of acid secreted in each aliquot was determined by titration with 0.01 N NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau, Switzerland). Blood samples were taken at the end of the experiment and plasma was collected for determination of antagonist concentrations. To determine the duration of inhibition of gastric acid secretion, an additional experiment was conducted where a single dose of JNJ-26070109 (3.65 μmol kg\(^{-1}\)) was administered 30 min after initiation of a continuous intravenous infusion of pentagastrin (39 pmol kg\(^{-1}\) min\(^{-1}\)). Gastric secretion aliquots were collected at 15 min intervals for the duration of the experiment to monitor the response to JNJ-26070109. Blood samples were collected throughout the experiment (0.25, 0.5, 1, 2 and 4 h) and the plasma concentration of JNJ-26070109 was determined as described above.

**Data analysis and statistics**

Values are represented as the mean ± S.E.M., n = 3-9. Statistical significance was determined using one-way analysis of variance (p < 0.05) followed by a Dunnett’s test for multiple comparisons.
For the gastric acid secretion experiments, responses were expressed as change in pH ($\Delta \text{pH}$) or as the total number of moles H$^+$ secreted over the investigated collection periods.

For the isolated in vitro stomach assay, individual $E/[A]$ curve data were fitted to the following Hill equation (eq.1), to provide estimates of midpoint location ([A]$_{50}$), maximal asymptote ($\alpha$) and Hill slope ($n_H$) parameters, where [A] is the agonist concentration and $E$ is the measured effect expressed as $\Delta \text{pH}$,

$$E = \frac{\alpha \cdot [A]^{n_H}}{[A]_{50}^{n_H} + [A]^{n_H}} \quad (1)$$

Analysis of competitive antagonism, expressed as pK$_B$ values, was performed by direct model-fitting to the Gaddum-Schild equation as described by Black et al. (1985). For in vivo experiments, ED$_{50}$ refers to the dose of compound that produced a half-maximal effect and EC$_{50}$ denotes the corresponding plasma concentration. For radioligand competition-inhibition curve data and for in vivo analysis of the inhibition of gastric acid secretion, concentration-response curve data were fitted to a four parameter general logistic function (eq. 2)

$$B = \frac{\alpha_{\text{min}} + \left(\alpha_{\text{max}} - \alpha_{\text{min}}\right)}{1 + 10^{\left(\log(10) \cdot [L] / n_H\right)}} \quad (2)$$

Results are presented as mean values ± S.E.M. Data between treatment groups were compared using Student’s two-tailed t-tests with $p < 0.05$ being considered statistically significant. All data were analyzed using the software package GraphPad Prism®, version 3.01 (GraphPad Software Inc., San Diego, CA).
Non-compartmental analysis of JNJ-26070109 pharmacokinetics was performed using WinNonlin Professional, version 4.0.1 (Pharsight Corporation, Mountain View, CA). Individual plasma concentrations and sample times for each animal were used in the analysis.

**Drugs**

JNJ-26070109 ((R) 4-Bromo-N-[1-(2,4-difluoro-phenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide) was synthesized in-house (see Woods et al., 2007 for description of synthesis) while YF476 ((R)-1-[2,3-dihydro-2-oxo-1-pivaloylmethyl-5-(2′-pyridyl)-1H-1,4-benzodiazepin-3-yl]-3-(3-methyl-phenyl)urea) was a generous gift from the James Black Foundation Ltd (see Figure 1 for structure of JNJ-26070109).
Results

Affinity and selectivity of JNJ-26070109 at Human, Rat, and Canine CCK receptors

The affinities of JNJ-26070109 (see Figure 1 for structure) and YF476 were determined across a number of different species using both radioligand binding (Table 1) and in vitro organ bath bioassays. The affinity of JNJ-26070109 for human CCK2 receptors ($\text{p}K_i = 8.49 \pm 0.13$) was approximately $\sim1200$-fold higher than for human CCK1 receptors ($\text{p}K_i = 5.41 \pm 0.06$; Figure 2A). The CCK2/CCK1 selectivity of JNJ-26070109 was lower for canine ($\sim336$-fold; Figure 2B) and rat ($\sim324$-fold; Figure 2C) receptors, which was largely a consequence of lower affinity for the rat and canine CCK2 receptor (all cell-based radioligand binding studies were $n=4$, conducted in triplicate). The human CCK1 receptor affinity of JNJ-26070109 was also confirmed in a binding assay prepared from a single sample of human gallbladder ($\text{p}K_i \sim 5.3$, conducted in sextuplet) and in an in vitro functional assay of guinea-pig gallbladder, where 60 min preincubation with $300 \mu M$ JNJ-26070109 had no significant effect on CCK-8S contractile $E/[A]$ curves (data not shown).

YF476 expressed approximately 10-fold higher affinity for human, rat and canine CCK2 receptors ($\text{p}K_i$ values $= 9.73, 9.50, 9.12$, respectively) than JNJ-26070109 (Table 1). However, the selectivity of YF476 for the CCK2 receptors in all
species was lower than that of JNJ-26070109 (human ~ 80-fold, dog ~20-fold, rat ~77-fold), due to the relatively higher affinity of YF476 for the CCK1 receptors.

The overall selectivity of JNJ-26070109 was also assessed in a panel of 50 other assays of pharmacological loci including GPCRs and ion channels (by Cerep, Inc). At a final assay concentration of 1 μM there was no significant effect in any of the assays (data not shown).

Inhibition of cholecystokinin-stimulated calcium flux by JNJ-26070109 at the human CCK2 receptor expressed in HEKZFP cells in the FLIPR assay
Sulphated cholecystokinin octapeptide (CCK-8S) stimulated calcium flux with a pA₅₀ of 7.9±0.03 (n=3, conducted in triplicate) at the human CCK2 receptor. The response was inhibited by JNJ-26070109 in a concentration-dependent manner and a pKᵦ value of 8.53±0.05 was estimated from these data (Figure 3). JNJ-26070109 behaved as a simple, competitive antagonist, as no change in maximal asymptote or Hill slope were observed over the concentration range evaluated and the Gaddum-Schild slope was not significantly different from unity (0.97±0.03).

Effect of JNJ-26070109 on pentagastrin-stimulated acid secretion in the lumen perfused, mouse stomach assay in vitro.
Pentagastrin, an established experimental surrogate for the hormone gastrin that retains the full biological activity of the hormone, produced a concentration-dependent increase in gastric acid secretion in the isolated mouse stomach assay ($p[A]_{50}=8.38\pm0.12$, $n_H=0.66\pm0.05$, $\alpha=0.29\pm0.03$ $\Delta \mathrm{pH}$, $n=10$). JNJ-26070109 (20-200 nM, $n=6-9$) produced a concentration-dependent rightward shift of the pentagastrin $E/[A]$ curve with no significant effect on basal acid secretion or the midpoint Hill slope and upper asymptote of the pentagastrin curves (Figure 4A). Analysis of the log $[A]_{50}$ values obtained in the absence and presence of JNJ-26070109 gave a Gaddum-Schild plot slope estimate (1.05±0.15) which was not significantly different from unity (Figure 4B). When the data were re-fitted to the Gaddum-Schild equation with the slope parameter constrained to unit value, a $pK_B$ value of 8.19±0.13 was obtained.

**In vitro pharmacokinetic analysis of JNJ-26070109 in Caco-2 cells and human hepatocytes**

JNJ-26070109 showed high absorption potential in this endothelial cell permeability assay (two separate experiments were conducted). Compound flux from the apical to basolateral side ($\text{PappA-to-B} = 15.9\pm0.5 \text{ cm s}^{-1}$, $n=2$) was nearly identical to flux measured in the reverse direction ($\text{PappB-to-A} = 17.3\pm1.2 \text{ cm s}^{-1}$, $n=2$) indicating that the compound was not subject to active efflux mechanisms. In isolated human hepatocytes, the quantity of JNJ-26070109 remaining after 15, 30, 60 and 120 min was determined to be 93, 58, 69 and 59%, respectively. These data indicate that the half life of JNJ-26070109 in this
system was greater than 2 h in this system. JNJ-26070109 was highly protein bound in human and rat plasma (human >99.9%, rat >99.9%). Slightly less protein binding (98.8% bound) was measured in dog plasma.

**In vivo pharmacokinetic analysis of JNJ-26070109 in rats and dogs**

JNJ-26070109 exhibited a small volume of distribution and clearance in both rats and dogs (see Table 2 for calculated and measured pharmacokinetic values and Figure 5 for a graphical representation of these data). The half life for JNJ-26070109 was similar between rats ($t_{1/2} = 1.8 \pm 0.3$ h, n=3) and dogs ($t_{1/2} = 1.2 \pm 0.1$ h, n=3). Oral administration of JNJ-26070109 showed the compound was well absorbed with a $T_{Max}$ of 1.3 h and 3.3 h in rat and dog, respectively. JNJ-26070109 was found to have a high oral bioavailability and there was no significant difference between rat and dog (73 % and 92 %, respectively).

**Analysis of the inhibition of gastric acid secretion by JNJ-26070109 in the anesthetized rat; Ghosh and Schild model**

Intravenous pentagastrin infusion produced a stable baseline of acid secretion for 4-6 h. The assay was validated by including omeprazole and YF476 which both inhibited pentagastrin-stimulated acid secretion with $pED_{50}$ values of $3.7 \pm 0.9$ (22 mmol kg$^{-1}$) and $7.1 \pm 0.1$ (80 nmol kg$^{-1}$), respectively (n=3). Intravenous bolus administration of JNJ-26070109 produced a dose-dependent inhibition of gastric acid secretion with a $pED_{50}$ value of $6.57\pm0.07$ (270 nmol kg; Figure 6A,
n=6). The corresponding plasma concentration-response curve was obtained from analysis of samples taken at the plateau of each response to JNJ-26070109 and from these data the concentration of drug in the plasma which produced a half maximal effect was calculated to be ~1 µM (pEC$_{50}$ = 6.0 ± 0.1; Figure 6B).

**Analysis of the inhibition of gastric acid secretion by JNJ-26070109 in the conscious rat, gastric fistula model**

Subcutaneous administration of pentagastrin (30 nmol kg$^{-1}$) increased acid secretion (Figure 7). The increase in acid secretion was maximal 1 hr after administration. Oral administration of JNJ-26070109 produced a dose-dependent inhibition of pentagastrin-stimulated acid secretion with an ED$_{50}$ value ~3 µmol kg$^{-1}$ and corresponding EC$_{50}$ value ~1.5 µM (pEC$_{50}$ = 5.82 ± 0.24; Figure 7A and C, n=6). There was no significant effect of 30 µmol kg$^{-1}$ JNJ-26070109 following oral administration on the secretory response to a near-maximal dose (30 µmol kg$^{-1}$) of histamine (data not shown).

**Analysis of the inhibition of gastric acid secretion by JNJ-26070109 in the conscious dog, gastric fistula model**

JNJ-26070109 inhibited pentagastrin-stimulated acid secretion in a dose-dependent manner (Figure 7B and D, n=6) with an ED$_{50}$ value of ~1.5 µmol kg$^{-1}$ and a corresponding EC$_{50}$ value, determined at the 90 min collection period, of 0.26 µM (pEC$_{50}$ = 6.58 ± 0.15, see Figure 7D).
The kinetic profile of gastric acid inhibition by JNJ-26070109 was further investigated in the dog by measuring the effect of a single intraduodenal dose of JNJ-26070109 (3.65 μmol kg⁻¹) on the steady-state stable response to a continuous near-maximal infusion of pentagastrin (39 pmol kg⁻¹min⁻¹). The maximum pharmacodynamic effect of JNJ-26070109 was achieved approximately 45 min after dosing. The subsequent reduction in response paralleled the plasma concentration profile of JNJ-26070109, returning to control values 4 h following dosing (Figure 8, n=6).
Discussion

CCK2 receptor antagonists could provide therapy for a number of different illnesses, including cancer, anxiety and disorders of gastric acid secretion. Indeed promising clinical data has been presented for some CCK2 receptor antagonists, including single dose administration of YF476 in the regulation of gastric acid secretion (Boyce et al., 2000a) and continuous infusion of JB95008 in the treatment of pancreatic cancer (Chau et al., 2006). However, despite the discovery and development of a number of CCK2 receptor ligands, there are no commercially available compounds for use in the clinic. Although not clearly established, it would appear that many compounds in this class have been discontinued due to a number of reasons including loss of efficacy upon repeat dosing (Boyce et al., 2000b) and poor and variable pharmacokinetics (Herranz et al., 2003). Therefore, a medicinal chemistry program was initiated with the goal of discovering novel chemical classes of antagonist with optimal biopharmaceutical properties, most notably high oral bioavailability and a robust relationship between plasma concentration and pharmacological effect (Allison et al., 2006, Woods et al., 2007; Rosen et al., 2008). This program resulted in the identification of JNJ-26070109. The in vitro pharmacology of this compound along with in vivo efficacy data are presented here.

The human CCK2 receptor binding affinity was determined in a system in which the level of CCK2 receptor expression in a human cell line was up-regulated by engineered transcription factors (see Morton et al., 2005).
system allows the CCK2 receptor to be investigated in its wild-type cellular context and, in the absence of an assay of human tissue, provides a receptor-expression system for the investigation of CCK receptor ligands. In this assay, JNJ-26070109 expressed a high affinity at the human CCK2 receptor \( (\text{pK}_i=8.49\pm0.13) \). JNJ-26070109 also expressed a high affinity at the cloned rat and dog CCK2 receptors, however, the affinity values estimated at these isoforms were lower than at the human CCK2 receptor (~3-fold lower at rat and 6-fold lower at dog CCK2 receptor). Species differences in the affinity of CCK receptor ligands have been previously documented. For example, a single amino-acid substitution in the CCK2 receptor has been shown to account for the reverse selectivity of the non-peptide antagonists L-365,260 and L-364,718 between dog and human CCK2 receptors (Beinborn et al., 1993). The differences in affinity observed in our study did not result in the reversal of selectivity for JNJ-26070109 in any species investigated. Indeed, JNJ-26070109 was shown to be highly selective for the CCK2 receptor across all species with the greatest fold-selectivity (>1200) observed at the human isoforms of the CCK receptors. Therefore, JNJ-26070109 represents one of the most selective CCK2 receptor antagonists identified to date.

The functional effects of JNJ-26070109 were evaluated in vitro, both in a cell-based calcium flux assay and in an isolated, lumen-perfused, mouse stomach assay. In both of these studies, JNJ-26070109 behaved as a surmountable, competitive antagonist with an equilibrium dissociation constants estimate
(pK_B~8.2 for the mouse and pK_B~8.5 for the human) consistent with the pK_i value generated for the human CCK2 receptor in the radioligand binding studies.

Gastric acid secretion assays were also utilized to assess the pharmacodynamic properties of this compound in vivo. In the rat, two models were employed such that the activity of the compound could be assessed following intravenous and oral dosing. These studies demonstrated that JNJ-26070109 inhibited pentagastrin-stimulated gastric acid secretion with a similar potency for both routes of administration. This is consistent with the high oral bioavailability estimated for this compound in the pharmacokinetic studies. Other CCK2 receptor antagonists have also been evaluated in this model. For example, YF476 was shown to inhibit pentagastrin-stimulated gastric acid secretion in anaesthetized rats with an ED_{50} value of ~0.01 μmol/kg for bolus intravenous administration (Takinami et al., 1997). This value is consistent with the 37-fold higher affinity of YF476 for the rat CCK2 receptor compared to JNJ-26070109 (pK_i values 9.55 and 7.89, respectively).

Pentagastrin-stimulated gastric acid secretion was also measured in conscious dogs following intraduodenal administration of JNJ-26070109. In these studies, JNJ-26070109 dose-dependently inhibited pentagastrin-stimulated acid secretion. Despite the ~3-fold lower affinity JNJ-26070109 expressed for the canine CCK2 receptor compared to the rat, the plasma concentration required to cause a half-maximal inhibition was ~3-fold lower in the dog than in the rat. The
reason for this is not clear but could be related to differences in plasma protein binding or in the distribution of the compound to the gastric mucosa between the species. In the canine model, the kinetic nature of this response was investigated further and the data obtained demonstrated that the time-dependent level of inhibition was paralleled by the plasma concentration profile.

The pharmacokinetic properties of JNJ-26070109 were evaluated in human in vitro assays and in vivo using both rats and dogs. These studies revealed that this compound was not subject to active transport in human Caco-2 cells and expressed a half-life greater than 2 h in isolated human hepatocytes. In vivo, JNJ-26070109 had high oral bioavailability in rats and dogs (%F=70-92 %) and appeared to have a moderate clearance and volume of distribution.

Overall, JNJ-26070109 behaved as a potent and selective CCK2 receptor antagonist and displayed a pharmacokinetic-pharmacodynamic profile that make it suitable for further evaluation in repeat dose, pre-clinical disease models and the clinic.
Authorship Contribution

Magda F. Morton. Participated in research design, conducted experiments, contributed new reagents or analytical tools, performed data analysis, wrote or contributed to the writing of the manuscript.

Terrance D. Barrett. Participated in research design, conducted experiments, contributed new reagents or analytical tools, performed data analysis, wrote or contributed to the writing of the manuscript.

Jamie Freedman. Participated in research design, conducted experiments, performed data analysis.

Lina Li. Participated in research design, conducted experiments, performed data analysis.

Michele C. Rizzolio. Participated in research design, conducted experiments, performed data analysis.

Clodagh E. Prendergast. Participated in research design, conducted experiments, performed data analysis.
Xiaodong Wu. Participated in research design, conducted experiments, performed data analysis.

Veronica Moreno. Participated in research design, conducted experiments, performed data analysis. Wrote or contributed to the writing of the manuscript.

Jayashree Pyati. Participated in research design, conducted experiments, performed data analysis.

Katherine Figueroa. Participated in research design, conducted experiments, performed data analysis.

Laurence Cagnon. Participated in research design, conducted experiments, performed data analysis.

Guy Lagaud. Participated in research design, conducted experiments, performed data analysis.

Luc Ver Donck. Participated in research design, conducted experiments, performed data analysis.
Etienne Ghoos. Participated in research design, conducted experiments, performed data analysis.

Brett Allison. Contributed new reagents or analytical tools.

Michael H. Rabinowitz. Contributed new reagents or analytical tools.

Nigel P. Shankley. Participated in research design, performed data analysis, wrote or contributed to the writing of the manuscript.
References


Footnotes

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Legends for Figures

Figure 1. Chemical structure of JNJ-26070109.

Figure 2. Inhibition of specific bound $^{\text{125}}$I-BH-CCK-8S (20 pM) by increasing concentrations of JNJ-26070109 at recombinant human (panel A), canine (panel B) and rat (panel C) CCK2 (closed symbols) and CCK1 (open symbols) receptors in different species. N=4 experiments each conducted in triplicate.

Figure 3. Inhibition of CCK-8S stimulated calcium flux by increasing concentrations of JNJ-26070109 at the human CCK2 receptor HEKZFP cells. Calcium flux was measured using FLIPR and the response expressed as percent (%) of maximum response in relative fluorescence units. Individual CCK-8S E/[A] curves in the presence of increasing concentrations of JNJ-26070109 are shown (panel A) and the corresponding Gaddum-Schild plot (panel B).

Figure 4. Pentagastrin-stimulated gastric acid secretion measured in the isolated mouse stomach in the absence and presence of increasing concentrations of JNJ-26070109. Closed circles show the vehicle control and the symbols show the log concentration of JNJ-26070109 tested (panel A). Acid secretion is represented by the change in pH from baseline (delta pH, y-axis) versus the concentration of pentagastrin added to the organ bath (x-axis). The
corresponding Gaddum-Schild plot is also shown (panel B). N=6-10 animals for each concentration of JNJ-26070109.

Figure 5. Pharmacokinetic profile of JNJ-26070109 in rats (panel A) and dogs (panel B) after intravenous (closed squares) and oral (open squares) administration. The plasma concentration of JNJ-26070109 (y-axis) was determined over different time intervals (x-axis). For the rat study, 2 μmol kg⁻¹ JNJ-26070109 was administered by both routes, whereas in the canine study 5.5 μmol kg⁻¹ JNJ-26070109 was dosed intravenously and 18.3 μmol kg⁻¹ JNJ-26070109 was administered orally. Data generated from 3 animals of each species.

Figure 6. Inhibition of pentagastrin-stimulated gastric acid secretion in the anesthetized rat by intravenous administration of JNJ-26070109 (0.01 to 10 μmol kg⁻¹). Acid secretion is represented by the change in pH from baseline (delta pH, y-axis) versus either the dose of JNJ-26070109 administered (panel A) or the plasma concentration of JNJ-26070109 (panel B). Data was generated from 6 animals.

Figure 7. Inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats and dogs by oral administration of JNJ-26070109. Basal acid secretion in vehicle control animals is shown by the white bar (panel A only), pentagastrin stimulated gastric acid secretion in vehicle treated animals is shown in the closed
bars and the inhibition of the pentagastrin stimulated response by JNJ-26070109 (0.3 to 30 μmol kg⁻¹ p.o.) is shown in grey bars. The total acid secreted is plotted versus the dose of JNJ-26070109 administered (panels A and B, rat and dog data respectively). For fitting the plasma concentration data the acid production in pentagastrin treated controls was defined as the no inhibition level and the acid production in vehicle treated animals was defined as the maximum inhibition possible. The percentage response was plotted versus plasma concentration of JNJ-26070109 (panels C and D, rat and dog data respectively). Data generated from 6 animals. The stars in panel A and B show a statistically significant reduction in acid production at the respective doses compared to pentagastrin alone (p<0.05).

Figure 8. The duration of effect of a single intraduodenal dose of JNJ-26070109 in chronic gastric fistula dogs. JNJ-26070109 at a dose of 3.65 μmol kg⁻¹ was administered into the duodenum at time 0 against a continuous infusion of pentagastrin (39 pmol kg⁻¹min⁻¹) and acid secretion was measured every 15 min (open circles represent H⁺ secretion relative to controls, left y-axis). The plasma concentration of JNJ-26070109 (closed squares, right y-axis) was measured throughout the study. Data generated from 6 animals.
Tables

Table 1. Summary of affinity values (pKᵢ) for JNJ-26070109 and YF746 determined in CCK1 and CCK2 receptor radioligand binding assays. [¹²⁵I]-CCK-8S was used as radioligand for the cell-based assays and [³H]-L-364,718 was used as radioligand for the human gallbladder assay. The estimated Hill slope parameters were not significantly different from unity in all cases. N=4 experiments each conducted in triplicate with the exception of the human gallbladder assay which was conducted once in sextuplet.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>pKᵢ value for JNJ-26070109</th>
<th>pKᵢ value for YF476</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK2</td>
<td>Human</td>
<td>8.49±0.13</td>
<td>9.73±0.12</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>7.99±0.08</td>
<td>9.50±0.05</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>7.70±0.14</td>
<td>9.12±0.07</td>
</tr>
<tr>
<td>CCK1</td>
<td>Human</td>
<td>5.41±0.06</td>
<td>7.83±0.08</td>
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<tr>
<td></td>
<td>Human Gallbladder</td>
<td>5.34</td>
<td>7.89±0.05*</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>5.48±0.09</td>
<td>7.62±0.06</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>5.17±0.08</td>
<td>7.82±0.13</td>
</tr>
</tbody>
</table>

*These data are taken from Morton et al., 2002.
Table 2. Summary of the pharmacokinetic properties of JNJ-26070109 after intravenous and oral administration to rats and dogs. Values are the mean ± S.E.M, n = 4 for rats and n = 3 for dogs

<table>
<thead>
<tr>
<th>Dose (µmol kg⁻¹)</th>
<th>Cmax (µM)</th>
<th>Tmax (h)</th>
<th>Vdss (L kg⁻¹)</th>
<th>AUC (µM•h)</th>
<th>T₁/₂ (h)</th>
<th>CL (L kg⁻¹h⁻¹)</th>
<th>%F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rat data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 – iv</td>
<td>NA</td>
<td>NA</td>
<td>0.19±0.05</td>
<td>33.3±8.3</td>
<td>1.8±0.3</td>
<td>0.08±0.03</td>
<td>NA</td>
</tr>
<tr>
<td>2 – po</td>
<td>6.4±0.8</td>
<td>1.3±0.4</td>
<td>NA</td>
<td>20.4±5.7</td>
<td>NA</td>
<td>NA</td>
<td>73±16</td>
</tr>
<tr>
<td><strong>Dog data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>5.48 - iv</td>
<td>NA</td>
<td>NA</td>
<td>0.51±0.05</td>
<td>17.4±1.5</td>
<td>1.2±0.1</td>
<td>0.32±0.003</td>
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<td>18.3 - po</td>
<td>9±1.8</td>
<td>3.3±0.7</td>
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<td>53.3±9.3</td>
<td>NA</td>
<td>NA</td>
<td>92±12</td>
</tr>
</tbody>
</table>
FIGURE 1
Figure 2

A. Human CCK receptors

B. Canine CCK receptors

C. Rat CCK receptors

% Specific bound

[JNJ-26070109]: log M

% Specific bound

[JNJ-26070109]: log M

% Specific bound

[JNJ-26070109]: log M
A. Agonist effect curve

B. Gaddum-Schild plot

FIGURE 4
FIGURE 6

A. Dose-response

B. Plasma concentration-response

\[ \Delta \text{pH} \]

-9 -8 -7 -6 -5

[JNJ-26070109]: log mol/kg

\[ \Delta \text{pH} \]

-8 -7 -6 -5

[Plasma JNJ-26070109]: log M