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Pharmacological Characterization of the Novel Histamine H_3-Receptor Antagonist SCH 79687

Robbie L. McLeod[1], Charlie A. Rizzo[1], Robert E. West Jr.[1], Robert Aslanian[2], Kevin McCormick[2], Matthew Bryant[3,4], Yunsheng Hsieh[3], Walter Korfmacher[3], Garfield G. Mingo[1], LoriAnn Varty[1], Shirley M. Williams[1], Neng-Yang Shih[2], Robert W. Egan[1] and John A. Hey[1]

[1] Allergy, [2]Chemistry, [3]Drug Metabolism and Pharmacokinetics,
Schering-Plough Research Institute, Kenilworth, NJ, USA 07033-0539

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Running Title: Pharmacological Profile of SCH 79687

Abbreviations;

EFS - electrical field stimulation
PPA – phenylpropanolamine
GP – guinea pig
CTM - chlorpheniramine
HSV - human saphenous vein
BP- blood pressure
CNS – central nervous system

Corresponding Author:

Robbie L. McLeod, Ph.D.

Allergy

Schering-Plough Research Institute

2015 Galloping Hill Road

Kenilworth, NJ 07033-0539

USA

Tel: (908) 740-3286

Fax: (908) 740-7175

E-mail: robbie.mcleod@spcorp.com

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Abstract

We present the pharmacological and phamacokinetic profiles of a novel histamine H_3-receptor antagonist, SCH 79687. The H_3-receptor binding K_i values for SCH 79687 were 1.9 nM and 13 nM in the rat and guinea pig (GP), respectively. The Ki values for SCH 79686 at histamine H_1 and H_2-receptors were greater than 1μM. SCH 79687 showed a 41- and 82-fold binding selectivity for the H_3-receptor over α_{2A}-adrenoceptors and imidazoline I_{2}-, and > 500-fold H_3-selectivity compared to over 60 additional receptors. The pA_2 value for SCH 79687 in the GP ileum electrical field stimulated (EFS) contraction was 9.6 ± 0.3. Similar H_3 antagonist activity was observed in the EFS cryopreserved and fresh tissue isolated human saphenous vein (HSV) assays (pK_b = 9.4 ± 0.3 and 10.1 ± 0.4). SCH 79687 (30 nM) did not block clonidine-induced inhibition of EFS-induced contractions in HSV.

SCH 79687 (ED_{50} = 0.3 mg/kg, i.v.) attenuated (R)-α-methylhistamine inhibition of sympathetic hypertensive responses in the GP. At the time of activity evaluation, the GP plasma SCH 79687 concentration was 25 ng/ml at the dose of 0.3 mg/kg i.v. In feline nasal studies, combined administration of SCH 79687 (3 mg/kg, i.v.) and the H_1-antagonist loratadine (3 mg/kg, i.v.), at individual doses that do not produce decongestion, inhibited the compound 48/80-induced congestion by 47%. The α-adrenergic agonist phenylpropanolamine (PPA; 1 mg/kg, i.v.) also attenuated compound 48/80 nasal responses by 42%. Unlike the H_3/H_1 combination that did not affect blood pressure (BP), PPA (1 mg/kg, i.v.) significantly increased BP compared to control animals by a maximum of 31 mmHg. Orally, SCH 79687 (10 mg/kg) plus loratadine (10 mg/kg) also produced decongestion without effects on BP. In pharmacokinetic studies, oral dosing with SCH 79687 in the rat (10 mg/kg) and monkey (3 mg/kg) achieved plasma Cmax and AUC values greater than 1.5 μg•hr/ml and 12.1 μg•hr/ml, respectively.

SCH 79687 is an orally active H_3-antagonist with a good pharmacokinetic profile that, in combination with an H_1-antagonist, demonstrates decongestant efficacy comparable to oral sympathomimetic decongestants but without hypertensive liabilities.

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Allergic rhinitis is a highly prevalent and chronic disease that affects a significant portion of the US population (Naclerio, 1991; Blaiss, 2000). Symptomatic of the disease are pruritis, sneezing, rhinorrhea, mucosal inflammation and nasal congestion (Turner and Forman, 1999; Corey et al., 2000). Mast cell histamine is a principal mediator in nasal allergic responses in sensitized people (Babe and Serafin, 1996; Baraniuk, 1998; Park and Baraniuk, 2002). Histamine exerts numerous local and systemic effects by activation of pharmacologically distinct histamine receptors, namely, H_1, H_2, H_3 and possibly through a newly discovered H_4-receptor (Hirschowitz, 1979; Leurs et al., 1995; Baroody and Naclerio, 2000; Nakamura et al., 2000; Nguyen et al., 2001; Alves-Rodrigues et al., 2001; Morse et al., 2001; Stark et al., 2001). Many of the allergic nasal responses to histamine such as mucus secretion, increased vascular permeability and pruritis are mediated by H_1-receptors (Babe and Serafin, 1996). A significant contribution of histamine H_2-receptors to nasal allergic responses has not been demonstrated and a role for H_4-receptors, which are found predominantly on inflammatory cells, in upper airway allergic responses remains to be identified.

Histamine H_3-receptors are widely distributed in the CNS and in the peripheral autonomic nervous system (Arrang et al., 1983; Coruzzi et al, 1991; Yanai et al., 1994; Gőthert et al., 1995). Peripheral H_3-receptors may serve as a target for the development of novel antiallergy drugs, in particular, mechanism-based decongestants (McLeod et al., 1999, 2001). These receptors prejunctionally modulate sympathetic neurotransmission and attenuate a variety of organ responses governed by sympathetic nervous system regulation (Koss and Hey, 1992; McLeod et al., 1993; Yu et al., 2001). Moreover, in vivo histamine H_3-receptors play a physiological role in the modulation of nasal vascular tone, a regulator of nasal patency (Bolser et al., 1994; McLeod et al., 2001). Additional, we have observed that in an experimental nasal congestion model that histamine H_3-antagonists when administered in combination with an H_1-antagonist can

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evoke decongestant activity in addition to the antiallergy effects that arise from sole blockade of H_1-receptors (McLeod et al., 1999).

In the present study we characterized the preclinical in vitro and in vivo pharmacological activity and pharmacokinetic profile of SCH 79687 (Aslanian et al., 2002), a novel, selective and potent histamine H_3-receptor antagonist. In addition, we studied the oral nasal decongestant effect of SCH 79687 (Figure 1) alone and in combination with the histamine H_1-antagonist loratadine in a feline experimental model of nasal congestion.

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Methods

Materials. N-(3,5-dichlorophenyl)-N'-[[4-(1H-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687 dihydrochloride), cimetidine and chlorpheniramine maleate were supplied by Chemistry, Schering-Plough Research Institute (Kenilworth, NJ). [^{3}H]N$^\alpha$-methylhistamine (81 Ci/mmole) was purchased from Dupont. (R)-α-methylhistamine dihydrochloride, thioperamide maleate, clonidine dihydrochloride, *l*-norepinephrine bitartrate, prazosin hydrochloride and compound 48/80 were obtained from Sigma-RBI (Natick, MA). Concentrated stock solutions of chlorpheniramine maleate, cimetidine, *l*-norepinephrine, clonidine and KCl were prepared in water. Concentrated stock solutions of SCH 79687 and thioperamide were prepared in dimethyl sulfoxide (DMSO) and either further diluted in DMSO (guinea pig ileum experiments) or water (human saphenous vein) before addition to the baths. Final concentrations of DMSO did not exceed 0.06% (GPI) or 0.01% (HSV) by volume in the bath. Sterile filtered heat inactivated fetal bovine serum, sucrose and KCl were obtained from Sigma (St. Louis, MO). For intravenous in vivo studies, loratadine was dissolved in 30% DMSO, 40% ethyl alcohol and physiological saline (0.9%) and given slowly over 2 min. SCH 79687 was dissolved in 30% DMSO and saline. All drugs doses refer to their respective free base. For oral cat decongestant studies, drugs were administered in a gelatin capsules (Size 0; Torpac Inc., Fairfield, NJ). All control animals were given appropriate vehicle controls.

Animal Care and Use. These studies were performed in accordance to the NIH GUIDE TO THE CARE AND USE OF LABORATORY ANIMALS and the Animal Welfare Act in an AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care) program.

Guinea-pig histamine H_3-receptor binding assay. Histamine H_3 receptor binding was performed according to West et al. (1990). Guinea pig brains were obtained frozen, thawed at room temperature and homogenized in ten volumes

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(w/v) of ice-cold 50 mM Tris HCl pH 7.5 buffer and disrupted with a Polytron (PTA 10 tip, 30 sec at setting 5). After low-speed centrifugation (10 min, 1000 xg), the supernatant was centrifuged ten min at 50,000 xg. The high-speed pellet was resuspended in the original volume of buffer, a sample was taken for protein assay (bicinchoninic acid, Pierce, Rockford, IL) and the suspension was centrifuged again at 50,000 xg. Pellets were removed and frozen at –80 ^{o} C until use.

Membrane (300 µg of protein) was incubated with [^{3}H]N$^{\propto}$-methylhistamine (0.5 nM) without or with inhibitor compounds in a total volume of 200 µl buffer. Nonspecific binding was determined in the presence of 10 µM thioperamide. Assay mixtures were incubated for 30 min at 30 ^{o} C in polypropylene, 96-well, deep-well plates then filtered through 0.3% polyethylenimine-soaked GF/B filters. These were washed three times with 1.2 ml of buffer, dried in a microwave oven, impregnated with Meltilex scintillant and were counted at 40% efficiency in a Betaplate scintillation counter (Wallac). Curves were fit to the guinea pig data for SCH 79687 with Prism nonlinear least-squares curve-fitting program (GraphPad Software, San Diego, CA). One- and two-site fits were tested.

Additional receptor binding characterization. SCH 79687 was run against a panel of 65 receptor binding assays (MDS Pharma Services, Taipei, Taiwan). Methods employed in all receptor assays were adapted from the scientific literature and are available upon request from MDS Pharma Services. SCH 79687 was tested in duplicate at 1 µM.

Histamine H_3-receptor antagonist activity in isolated guinea pig ileum and human saphenous vein. Discarded human saphenous veins (HSV) from coronary artery bypass graft patients (male and female, age = 53 - 80 years, Hackensack University Medical Center Institute for Biomedical Research, Hackensack, NJ) stored and shipped in 4 ^{o} C heparinized autologous blood were received within 30 hr of removal. HSV rings (3 - 7 mm diameter X 5 mm) were used fresh upon arrival or cryopreserved. Cryopreservation and thawing were as

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previously described in Valentine et al. (1999) except for a 10 min cryofreezing container equilibration at 4 ^{o}C before its transfer to -70 ^{o}C.

The general methods used for the isolated guinea pig ileum (GPI) and HSV assays, including guinea pig strain, sex and size, were as described in Valentine et al. (1999). The assays were performed in the presence of chlorpheniramine maleate (CTM, 1 µM) alone (GPI) or combined with cimetidine (1 µM, HSV) to block H_1 and H_2-mediated effects respectively (Leurs et al., 1995).

Whole longitudinal GPI segments (2 cm) equilibrated at 0.3 g passive tension were contracted repetitively (1 min intervals) using submaximal electrical field stimulation (EFS), pulse duration and voltage adjusted to attain 60% to 80% of the reference contraction to 25 Hz, 8 V, 1 ms pulse duration and 1 s train/min. Antagonist was added five min prior to the initiation of repetitive submaximal EFS trains. After the first stimulus train, rising cumulative additions of (R)-α-methylhistamine (1 nM - 100 µM, half-log increments) were performed one min before each succeeding stimulus train.

Fresh and cryopreserved HSV rings equilibrated at 1.0 g passive tension were contracted using submaximal EFS (16 Hz, 1 ms pulse duration and submaximal voltage). A 45 min antagonist equilibration preceded a series of 30 sec train EFS-induced contractions performed at 15 min intervals. After the control EFS-train, rising cumulative additions of (R)-α-methylhistamine (0.01 nM - 100 µM, log increments) were performed 10 min before each succeeding stimulus train. Prazosin (1 µM), an α_1-adrenoceptor antagonist (McGrath et al. 1989), applied 10 min before the final stimulus supplied the maximum inhibition of the α-adrenergic component of the EFS response used to normalize (R)-α-methylhistamine responses. For studies of SCH 79687 (30 nM) effects on prejunctional α_2-adrenergic receptor modulation of EFS, clonidine, an α_2-adrenergic agonist (McGrath et al., 1989), replaced (R)-α-methylhistamine. To evaluate SCH 79687 effects on postjunction-mediated contractility, cumulative applications of *l*-norepinephrine (0.1 - 100 µM), a nonspecific α-adrenergic agonist (McGrath et al., 1989), and an application of KCl (80 mM) preceded a 1

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hr equilibration with 3 or 10 μM SCH 79687 and a repeat of the *l*-norepinephrine and KCl applications.

In vivo studies

Histamine H_3-antagonist activity in the guinea pig. The procedure for evaluating the in vivo histamine H_3-receptor activity has been previously described (Hey et al., 1992). The in vivo histamine H_3 antagonist activity of SCH 79687 was evaluated by measuring its ability to block the inhibitory effects of (R)-α-methylhistamine on the sympathetic hypertensive responses evoked by electrical stimulation of the medulla oblongata. Male Hartley guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and surgically prepared for catheterization of the trachea, jugular vein and carotid artery. Animals were mechanically ventilated (tidal volume = 4 ml, frequency = 45 breaths/ min) and paralyzed with gallamine triethiodide (2 mg/kg, i.v.) and pretreated with ipratropium bromide (10 μg/kg, i.v.) to block cholinergic cardiopulmonary responses. Animals were instrumented for measurement of blood pressure, heart rate and pulmonary insufflation pressure. All physiological parameters were recorded continuously on a physiograph. Animals were positioned in a stereotaxtic apparatus and implanted with electrodes for stimulation of medullary cardiopressor areas. The stimulation parameters used were: 32 Hz, 3-5 sec trains, 0.5 – 1.5 ms square pulses and varying intensities from 25 – 100 μA. The cardiopressor responses to CNS stimulation were evaluated before and after the test drug (or vehicle). SCH 79687 (0.03 – 3.0 mg/kg, i.v.) was given 5 min before (R)-α-methylhistamine (0.3mg/kg, i.v.). Peak cardiopressor responses were recorded to each stimulus intensity (25 – 100 μA) approximately 30 min after (R)-α-methylhistamine was given. The dose of (R)-α-methylhistamine was chosen based on its ability to produce maximal H_3 receptors mediated inhibition of CNS-induced hypertension (Hey et al., 1992). For determination of the inhibitory effect the stimulus intensity of 75 μA was selected because it elicits consistent, submaximal increases in blood pressure.

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Effect of SCH 79687 plus loratadine on increases in nasal resistance due to compound 48/80 in the cat. Acoustic rhinometry measurements of evaluating decongestant activity in anesthetized cat has been previously described (McLeod et al., 1999). Briefly, pentobarbital (35 mg/kg, i.p.) anesthetized cats were mechanically ventilated (volume = 25 ml, rate = 20 breaths/minute) with room air. The left nostril was sealed with Reprosil (Dentsply International Inc., Milford, DE). A cuffed endotracheal tube was advanced from the upper esophagus to the nasopharynx. A constant flow (1.7 L/min) of air was passed through the right nasal airway via the endotracheal tube. Nasal pressure values were converted to nasal resistance using the formula: Resistance = pressure/flow. Continuous blood pressure measurements were recorded on a Grass chart recorder.

The effects of combined H_1 and H_3-receptor blockade with loratadine (3 mg/kg, i.v.) and SCH 79687 (3 mg/kg, i.v.), loratadine alone, SCH 79687 alone, phenylpropanolamine (PPA; 1 mg/kg, i.v.) or vehicle were studied on the increases in nasal resistance produced by exposure to compound 48/80 (1%, aerosolized for 45 sec). The doses of loratadine (3 mg/kg, i.v.) and PPA (3 mg/kg, i.v.) where chosen to match previously validated doses of these drugs in the cat nasal congestion model (McLeod et al., 1999). Similarly, the dose of SCH 79686 was comparable to doses of thioperamide previously evaluated in the cat (McLeod et al., 1999, 2001). Drugs were given 30 min before administration of compound 48/80. Pharmacological responses were observed 40 min after drug treatment.

Effect of SCH 79687 plus loratadine on decreases in nasal cavity volume due to compound 48/80. Estimates of nasal volumes were determined according to the methods of McLeod et al. (1999) in the anesthetized cat using acoustic rhinometry equipment purchased from NADAR (Aarhus, Denmark). Sound waves produced from a spark generator were propagated down a rigid wave tube and entered the nasal cavity through an airtight 3.2 cm nosepiece. Reflected sound waves from the nose were amplified and recorded. The sampling frequency was 100 kHz. The data obtained were converted to area-

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distance function curves and were used to provide estimates of cross-sectional areas and nasal volumes. The distance measured from the nostril opening into the nasal cavity was 3.0 cm and was based on measurements of cast impressions made of the cat nasal passageways.

The effect of either the combination of loratadine (10 mg/kg, p.o.) and SCH 79687 (10 mg/kg, p.o.) or loratadine alone, SCH 79687 alone, PPA (10 mg/kg, p.o.) or vehicle given 2 hr before compound 48/80 (1.0%, 50 µl) was instilled into the left nares was evaluated. The right nares was given saline. Effects on the ratios of the volumes of the left to right nasal passage were evaluated at 3 hr after drug treatment.

Pharmacokinetic studies

Phamacokinetic profile in the guinea pig. A separate study was conducted in guinea pigs using the SCH 79687 ED_{50} to determine systemic exposure at this dose. Blood samples were collected 1, 5, 15, 20, 30 and 40 min post i.v. administration and then centrifuged to isolate plasma.

Phamacokinetic profile in the rat. Four male Sprague-Dawley rats were dosed p.o. at 10 mg/kg using a 0.4% (w/v) methylcellulose suspension of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.5, 1, 3, 5, 7 and 24 hours post dose. After settling on ice, plasma was isolated and placed into sample tubes. The tubes were stored at –20 ^{O}C until assayed via HPLC-API/MS/MS.

Pharmacokinetic profile of SCH 79687 administered to monkeys. Four male cynomolgus monkeys were dosed p.o. at 3 mg/kg using a 0.4% (w/v) methyl cellulose suspension of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.25, 0.5, 1, 2, 4, 6, 7.5, 12 and 24 hours post dose. Plasma was isolated and placed into

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sample tubes. The tubes were stored at –20 ^{o}C until assayed via HPLC-API/MS/MS.

Two male cynomolgus monkeys were dosed i.v. at 3 mg/kg using a 20% (w/v) 2-hydroxypropyl-β-cyclodextrin solution of SCH 79687 as the micronized crystalline dihydrochloride salt. Blood samples were collected into tubes containing heparin at 0.12, 0.25, 0.5, 1, 2, 4, 6, 7.5, 12 and 24 hours post dose. After settling on ice, plasma was isolated and placed into sample tubes. The tubes were stored at –20 ^{o} C until assayed via HPLC-API/MS/MS.

HPLC-API/MS/MS assay of plasma samples. Plasma samples were assayed for concentration of SCH 79687 using the technique of high performance liquid chromatoraphy-atmospheric pressure ionization tandem mass spectrometry (HPLC-API/MS/MS) as previously described (Bryant et al., 1997). Plasma (40 µl) was added to a microcentrifuge tube and subjected to protein precipitation with 100 µl of acetonitrile containing 0.2 ng/µl of an internal standard, SCH 66336-(4-{2-[4-(3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2b]pyridin-11-yl)-piperidin-1-yl]-2-oxo-ethyl}-piperidine-1-carboxylic acid amide; Liu et al., 1998). After vortexing for 30 sec and centrifugation at 12,000 x g for 8 min, the supernatant was transferred into HPLC injection vial. The HPLC system consisted of a Shimadzu LC-10AD pump and Perkin Elmer series 200 autosampler. Chromatographic separation of SCH 79687 and the internal standard was achieved with an Inertsil ODS-2 column (4.6 x 50 mm) using an isocratic solvent system containing 50% methanol in water (4 mM ammonium acetate) at a flow rate of 0.8 ml/min. The effluent from the HPLC system was connected directly to a PE-Sciex API 365 triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface. Multiple reaction monitoring was utilized for quantitation of SCH 79687 (MH^+ m/z 375 to a fragment ion m/z 171). Calibration samples (5 to 5,000 ng/ml) were prepared by spiking drug-free guinea pig plasma with known concentrations of SCH 79687 and then processed with the study samples. The calibration curve was constructed using least squares linear regression with $1/x^2$ weighting and

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utilized peak area ratios of the analyte and internal standard. Quality control samples at three specified concentrations were also run with the study to ensure accuracy of the calibration curve.

Data analysis and statistics. Contractions were measured as g tension increase over baseline, normalized as % reference EFS response for GPI EFS-induced contraction and as % KCl (80 mM) response for contractions to *l*-norepinephrine. The inhibition of EFS-induced contraction, normalized as % (R)-α-methylhistamine maximum (GPI) or % prazosin response (HSV), represented prejunctional agonist activity. Agonist EC_{50} (half-maximal concentration) was estimated using linear regression analysis of individual agonist concentration-response curves and EC_{50} or pD_2 ($-\log_{10}$ of the EC_{50}) was used to express potency. H_3-antagonist activity was represented by shift in the agonist EC_{50}, from which an agonist dose ratio (DR = A'/A, where A' and A are the EC_{50} values estimated in the presence and absence of the antagonist, respectively, was calculated (Tallarida, 1988). Antagonist affinity was estimated as pA_2 ($-\log_{10}$ of the antagonist molar concentration that produces a DR = 2) or apparent pK_b (= $-\log_{10}$ of K_b) (Tallarida and Murray, 1981;Tallarida, 1988). The pA_2 was calculated using Analysis I: Schild Plot of Tallarida and Murray (1981) and individual dose ratios from antagonist concentrations that yielded mean DR ≥ 2. Apparent K_b was estimated using K_b = [B]/(A'/A-1), where [B] is the concentration of antagonist tested (Tallarida, 1988) and individual dose ratios ≥ 2 from statistically active antagonist concentrations. Statistical significance was taken as $p < 0.05$ using a Kruskal-Wallis nonparametric multiple group analysis and/or a Mann-Whitney-U two-group analysis comparing control and treated EC_{50} values.

The nasal cavity volume data were expressed as the ratio of the volume of left treated nares versus the right untreated nares (McLeod et al., 1999). Values displayed in the table and the figures represent the MEAN ± SEM. For all in vivo studies, data were evaluated using a Kruskal-Wallis analysis in conjunction with a Mann Whitney-U. Statistical significance was set at $p < 0.05$.

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Results

Receptor binding profile. Competition of SCH 79687 versus [^{3}H]N$^{\propto}$-methylhistamine binding to guinea pig brain disclosed high-affinity inhibition of binding to an apparently single site. The K_i value for SCH 79687 at guinea pig was 13 ± 6. A Similar guinea pig K_i affinity was obtained for thioperamide (12 ± 6 nM). Table 1 lists the K_i values for SCH 79687 binding to a variety of receptor systems (MDS Pharma Services Counterscreen). In this analysis, SCH 79687 showed 41 and 82-fold selectivity for the H_3-receptor over the $\alpha_{2A\ (human)}$-adrenergic- and imidazoline $I_{2\ (rat)}$-receptor, respectively, and > 500-fold higher affinity for the H_3-receptor compared to 65 additional receptors (data not shown).

Histamine H_3-receptor antagonist activity in isolated guinea pig ileum and human saphenous vein. (R)-α-methylhistamine inhibited EFS-induced cholinergic contractions of isolated GPI, demonstrating mean EC_{50} = 8.4 ± 0.7 nM (pD_2 = 8.1, n = 61). The maximal inhibition by (R)-α-methylhistamine was nearly complete in this assay, amounting to 96.0 ± 1.0% of the baseline EFS response in a representative sample of tissues (n = 35, data not shown). SCH 79687 and thioperamide dose dependently inhibited (R)-α-methylhistamine activity, demonstrating pA_2 estimates in the sub to low nanomolar range (Table 2). Schild plot slopes were - 0.94 ± 0.25 (95% confidence limits -1.5 to -0.4), and - 0.8 ± 0.11 (95% confidence limits - 1.0 to - 0.5) for SCH 79687 and thioperamide, respectively.

(R)-α-methylhistamine inhibited EFS-induced sympathetic contractions of isolated HSV. Mean (R)-α-methylhistamine EC_{50} was 0.5 ± 0.2 nM (pD_2 = 9.3, n = 21) in cryopreserved tissue and 2.4 ± 1.7 nM (pD_2 = 8.6, n = 11) in fresh tissue. The potency of (R)-α-methylhistamine in fresh and cryopreserved HSV was not significantly different. In contrast to GPI, maximal (R)-α-methylhistamine inhibition of EFS was partial in HSV, amounting to 42.2 ± 3.2 and 29.1 ± 2.5% inhibition in samples of fresh and cryopreserved tissue respectively (n • 19, data

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not shown). In the same fresh and cryopreserved tissue samples respectively, prazosin (1 µM) produced 59.7 ± 5.7 and 49.9 ± 4.0% inhibition, thus maximal (R)-α-methylhistamine inhibition represented 71 and 58% of the prazosin-sensitive portion of the EFS twitch response. SCH 79687 and thioperamide dose-dependently inhibited (R)-α-methylhistamine responses and exhibited sub to low nanomolar pK_b estimates in cryopreserved HSV (Table 2). A pK_b of 10.1 ± 0.4 was estimated for SCH 79687 against (R)-α-methylhistamine in fresh HSV.

The specificity of SCH 79687 for the H_3-receptor was also investigated using pre- and post-junctional mediated contractions of cryopreserved HSV. Clonidine inhibited EFS-induced contractions of HSV (mean pD_2 = 10.4 ± 0.3, n = 6). The maximal clonidine inhibition of EFS twitch (28.8 ± 4.4% inhibition, n = 6) was similar to the maximal inhibition obtained with (R)-α-methylhistamine in this tissue. No significant inhibition of clonidine modulation of EFS contractions was seen with 30 nM SCH 79687 (n = 6, data not shown). In addition, SCH 79687 (≤ 10 µM) demonstrated no effects on the baseline tone or *l*-norepinephrine (pD_2 = 6.2 ± 0.3, n = 3)- and KCl (80 mM)-induced contractions of cryopreserved HSV (n = 3 - 4, data not shown).

Activity and pharmacokinetic profile of intravenous SCH 79687 in the guinea pig. (R)-α-methylhistamine (0.3 mg/kg, i.v.) inhibited sympathetic hypertensive responses evoked by stimulation of the medulla oblongata by 33 ± 4% (n=12). Figure 2 shows that intravenous SCH 79687 produced a dose dependent attenuation of the blood pressure effects of (R)-α-methylhistamine (ED_{50} = 0.28 mg/kg, n=5-6 animals per group). In a separate study to determine the pharmacokinetics of the compound at this dose, plasma samples were quantified at specified times after i.v. administration of SCH 79687. Mean plasma concentration of SCH 79687 (0.28 mg/kg, i.v.) at 1, 5, 15, 20, 30 and 40 min after i.v. dosing are shown in Table 3.

Nasal decongestant effect of SCH 79687 in combination with loratadine. Figure 3 displays the decongestant actions of combined H_1 and H_3 blockade with

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loratadine and SCH 79687. Loratadine (3 mg/kg, i.v.) administered together with SCH 79687 (3 mg/kg, i.v.) significantly blocked the increase in nasal resistance produced by aerosolized compound 48/80 (1%). Loratadine (3 mg/kg, i.v.) and SCH 79687 (3 mg/kg, i.v.) given alone did not alter nasal responses to compound 48/80. PPA (1 mg/kg, i.v.) decreased nasal resistance but also produced a pronounced increase in mean arterial blood pressure (Figure 3b). Loratadine plus SCH 79687 had no effect on blood pressure compared to control animals. The oral decongestant activity of combined loratadine and SCH 79687 is shown in figure 4. Loratadine (10 mg/kg, p.o.) given together with SCH 79687 (10 mg/kg, p.o.) inhibited the decrease in nasal cavity volume due to nasal instillation of compound 48/80. Similarly, PPA (10 mg/kg, p.o.) inhibited the nasal effects of compound 48/80. Loratadine and SCH 79687 given alone had no effect on compound 48/80-induced changes in nasal geometry. However, PPA significantly increased systolic blood pressure (157 ± 5, n=4) compared to control animals (111 ± 11 mm Hg, n=5). The blood pressure in cats treated with a combination of loratadine (10 mg/kg, p.o.) and SCH 79687 (10 mg/kg, p.o.) was 114 ± 12 mm Hg (n=5) and was not different from vehicle treated animals.

Phamacokinetic profile of SCH 79687 in the rat. The four rats showed similar (PK) profiles. The mean C_{max}, T_{max} and AUC values were 1.5 µg/ml, 4.5 hours and 18.1 µg.hr/ml, respectively. The mean PK profile for the four rats is shown in Figure 5. After a single p.o. dose of 10 mg/kg, the rats still showed measurable levels of SCH 79687 at 24 hours post administration.

Pharmacokinetic profile of SCH 79687 administered to monkeys. The four monkeys dosed p.o. showed similar PK profiles. The mean C_{max}, T_{max} and AUC values were 1.7 µg/ml, 4.4 hours and 12.6 µg.hr/ml, respectively. The two monkeys dosed i.v. were very well matched in their PK profile. The mean AUC and $t_{1/2}$ values were 24.1 µg.hr/ml and 2.5 hours, respectively. The mean PK profiles for the monkeys are shown in Figure 6. After a single p.o. dose of 3

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mg/kg, the monkeys still showed measurable levels of SCH 79687 at 24 hours post-dose.

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Discussion

SCH 79687 is a novel, selective, orally active histamine H_3-antagonist. SCH 79687 binds to H_3-receptors with high affinity. We found that SCH 79687 displayed a weaker binding in the guinea pig (Ki = 13 nM) compared to the rat (Ki = 1.9 nM). Differences in H_3-receptor binding affinities among difference species, including humans, are not unexpected (Lovenberg et la., 2000). In point of fact, previous studies have shown that imidazole containing H_3-receptor antagonists including thioperamide, GT-2016 and iodophenpropit typically display less potency against human H_3 receptors compared to H_3 receptors from other species (Ireland-Denny et al., 2001). Nonetheless, SCH 79687 has very weak or no affinity for other receptors including histamine H_1, H_2, adrenergic and muscarinic receptors. We also found that SCH 79687 showed dose-dependent antagonist activity in vitro against (R)-α-methylhistamine in the isolated fresh GPI and isolated fresh and cryopreserved HSV functional H_3-bioassays. The selective histamine H_3 receptor antagonist thioperamide demonstrated similar H_3 receptor antagonist potency to SCH 79687 in HSV. In the GPI thioperamide but was approximately 6-fold weaker than SCH 79687. Also in the GPI the Schild analysis was consistent with competitive blockade of (R)-α-methylhistamine by SCH 79687 and thioperamide at the concentrations tested. The estimated pA_2 for thioperamide against (R)- α-methylhistamine is similar to previous H_3 receptor pA_2 estimates reported for this compound in GPI (Menkveld and Timmerman 1990; Rizzo et al., 1995). Taken together, these findings confirm that SCH 79687 is a functional histamine H_3-receptor antagonist in vitro.

In guinea pig studies, SCH 79687 inhibited (R)-α-methylhistamine-induced attenuation of electrically provoked hypertensive responses in a dose dependent fashion. Inhibition of (R)-α-methylhistamine-induced depression of sympathetic hypertensive responses in the guinea pig is a method used to characterize the H_3-antagonist activity in vivo (Hey et al., 1992). In these studies we found that SCH 79687 (ED_{50} = 0.28 mg/kg, i.v.) displays potency comparable to the standard H_3-antagonist, thioperamide (ED_{50} = 0.4 mg/kg, i.v.). Additional

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evidence for the in vivo H_3-antagonist activity of SCH 79687 is provided by the feline congestion studies. We have previously demonstrated that the combination of a histamine H_3-antagonist with an H_1- antagonist produces nasal decongestant activity (McLeod et al., 1999, 2001). This study demonstrates that combination blockade of H_1- and H_3-receptors with oral or intravenous loratadine and SCH 79687 produces decongestant activity in the cat equivalent to the α-agonist decongestant, phenylpropanolamine. On the other hand, in contrast to phenylpropanolamine, which produces a significant hypertensive effect after oral administration, SCH 79687 plus loratadine did not alter systemic blood pressure. It is important to note that the nasal decongestant effect of combined SCH 79687 and loratadine are not likely due to either central blockade of either histamine H_1- and/or H_3-receptors. Loratadine is a second generation antihistamine that does not cross the blood brain barrier. Similarly, based on our plasma/brain pharmacokinetic analysis, SCH 79687 does not enter the CNS to a significant extent. After a 10 mg/kg oral dose in rats, the ratio of the drug concentration (AUC over 6 hours) in the brain relative the concentration in the plasma was 0.02, indicating that SCH 79687 does not penetrate the blood brain barrier to a significant extent. Moreover, at the oral dose tested in the cat (10 mg/kg), SCH 79687 did not elicit behavior effects indicative of central histamine H_3-receptor blockade (i.e. excitation, heightened arousal). Taken together, our studies suggest that the decongestant activity of SCH 79687 plus loratadine is likely due to peripheral activity at the level of the nasal mucosal blood vessels and the sympathetic nerves that innervate them. We proposed that during a nasal allergic reaction, mast cell-derived histamine stimulates prejunctional H_3-receptors to produce a dilation of blood vessels in the nose contributing to nasal congestion (McLeod et al., 2001). This histamine H_3-receptor mediated activity is in addition to the stimulation of postsynaptic H_1-receptors that elicit plasma extravasation, vasodilation and mucus secretion In support of this hypothesis, a recent study by Varty and Hey (2002) demonstrated that activation of histamine H_3-receptors inhibited neurogenic sympathetic vasoconstrictor responses in isolated pig nasal turbinates.

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The mean plasma concentration of SCH 79687 (Table 3) in the guinea pig efficacy model at the ED_{50} (0.28 mg/kg, i.v.) was found to be 25 ng/ml at 30 minutes post-dose when activity was still observed. These data provide a minimum plasma level for SCH 79687 that will be needed for activity. The pharmacokinetics in both the rat and the monkey showed a good plasma profile for SCH 79687 after oral dosing with levels above 25 ng/ml at 12 hours (see Figures 5 and 6) and measurable levels at 24 hours. These data demonstrate that SCH 79687 has favorable PK properties that would allow for BID oral dosing at moderate dose levels.

Interestingly, SCH 79687 demonstrated moderate binding to rat imidazoline I_2 receptors (K_i = 155 nM) and human α_{2a}-adrenoceptors receptors (K_i = 78 nM). A role for imidazoline I_2-receptors in the modulation of nasal patency has not been established. Presynaptic imidazoline receptors that modulate release of norepinephrine from post-ganglionic sympathetic nerves innervating the cardiovascular system have been identified (Molderings et al,, 1997). However, these receptors are distinct from the rat I_2-receptor to which SCH 79687 binds. We have previously demonstrated that activation of α_2-adrenergic receptors with drugs such as BHT-920 produced nasal decongestion in the cat (McLeod et al., 2001). However, the observation that SCH 79687 did not alter the inhibitory actions of clonidine on EFS-induced HSV contractions or affect baseline HSV tone suggest that α_{2}-adrenoceptors are not activated in vitro at the concentration presently studied. Moreover, in the feline congestion model, SCH 79687 administered alone did not significantly increase nasal patency or produce changes in blood pressure as would be expected with a functionally active α-adrenergic receptor ligand. Consequently, we have demonstrated that the combination of the selective histamine H_3 antagonist SCH 79687 and loratadine produces decongestant activity in the cat by a mechanism that eliminates the untoward side effects associated with sympathomimetic decongestants.

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Legend for Figures

Figure 1. Chemical structure of SCH 79687 (N-(3,5-dichlorophenyl)-N'-[[4-(1H-imidazol-4-ylmethyl)phenyl]- methyl]-urea).

Figure 2. Histamine H_3-receptor antagonist activity in the guinea pig. Figure illustrates the intravenous antagonist activity of SCH 79687 (0.03 – 3 mg/kg, i.v.) on (R)-α-methylhistamine inhibitory effects of electrically-induced hypertensive responses. Each value represents the Mean ± SEM of 5-6 animals per group (*p < 0.05 compared to control).

Figure 3. Effect of SCH 79687 in combination with loratadine on nasal resistance and blood pressure in the cat. Panel A displays the effect of vehicle, SCH 79687 (3 mg/kg, i.v.) alone, loratadine (3 mg/kg, i.v.) alone, phenylpropanolamine (PPA; 3 mg/kg) and SCH 79687 plus loratadine on aerosolized compound 48/80 (1%)-induced increases in nasal resistance. Panel B shows the effect of these treatments on mean arterial blood pressure. Each bar represents the Mean ± SEM of 6-7 animals per group (*p < 0.05 compared to compound 48/80 alone; n=9).

Figure 4. Effect of SCH 79687 in combination with loratadine on feline nasal cavity volume. Figure displays the oral effects of vehicle, SCH 79687 (10 mg/kg) alone, loratadine (10 mg/kg) alone, phenylpropanolamine (PPA; 10 mg/kg) and SCH 79687 plus loratadine on topical compound 48/80 (1%)-induced decreases in nasal cavity volume. Shown are the effects of these treatment regimens at 2 hr (immediately before compound 48/80 exposure) and 3 hr after oral administration. Each bar represents the Mean ± SEM of 4-5 animals per group (*p < 0.05 compared to compound 48/80 alone group at 2hr; $^{+}$p < 0.05 compared to compound 48/80 alone group at 3 hrs).

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Figure 5. Pharmacokinetic Profile of SCH 79687 in the rat. Figure illustrate the mean plasma concentration of SCH 79687 (10 mg/kg; n=4) after oral administration.

Figure 6. Pharmacokinetic Profile of SCH 79687 in the monkey. Shown are the mean plasma concentration of SCH 79687 after oral (n=4) and i.v. (n=2) administration.

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Footnotes

[4]Present address; Pharmacokinetics and Drug Metabolism, Bayer Corporation. West Haven, CT, USA 06516

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Table 1

Receptor binding characterization of SCH 79687*

Receptor target	Ki
histamine H_3 (rat)	1.9 nM
histamine H_1 (guinea pig)	> 1 µM
histamine H_2 (guinea pig)	> 1 µM
adrenergic α_{2A} (human)	78 nM
imidazoline I_2 (rat)	155 nM

* MDS Pharma Services Counterscreen profile

Please note that SCH 79687 did not display significant activity in the following MDS Pharma enzymes and receptor binding assays: acetylcholinesterase, carbonic anhydrase, choline acetyltransferase, myeloperoxidase, phosphodiesterase (II, III, IV, V), calpain, protease – cathepsin G, collagenase IV, protease – elastase, neutral endopeptidase, EGF receptor Tyr kinase, HER2 tyrosine kinase, CD45 Tyr phosphatase, protein phosphatase, PTP1B, protein phosphatase, PTP1C, adenosine A_3, adrenergic α_{1A}, $\alpha_{2C,}$ β_1, β_2, β_3, bradykinin B_1, calcitonin, Ca^{++} channel (L,N), cannabinoid (CB_1, CB_2), cholecystokinin (CCK_A, CCK_B), dopamine (D_1, D_2, D_3, D_4, D_5) endothelin ET_B, epidermal growth factor, glucocorticoid, interferon-γ, interleukin-6, interleukin-8, leukotriene B_4, muscarinic (M_1, M_2, M_3, M_4, M_5), neuropeptide Y_1, K+ channel (K_A), serotonin (5-HT_{1A}, 5-HT_6, 5-HT_7), sigma (σ_1,σ_2) tachykinin (NK_1, NK_2), vasoact. intest. pep. (VIP_1).

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Table 2

Histamine H_3 antagonist activity in the guinea pig ileum and cryopreserved isolated human saphenous vein

Compound	Guinea pig ileum pA_2	Human saphenous vein pK_b-estimate
SCH 79687	9.6 ± 0.3	9.4 ± 0.3
thioperamide	8.8 ± 0.24	8.9 ± 0.5

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Table 3

Mean Plasma Concentration of Intravenous SCH 79687 in Guinea Pigs at the ED_{50} (0.28 mg/kg)

Time post dose	(n)	Mean plasma concentration (ng/ml)	Coefficient of variation (%)
1	5	644	43
5	5	112	42
15	5	39	43
20	4	30	43
30	5	25	61
40	5	18	67

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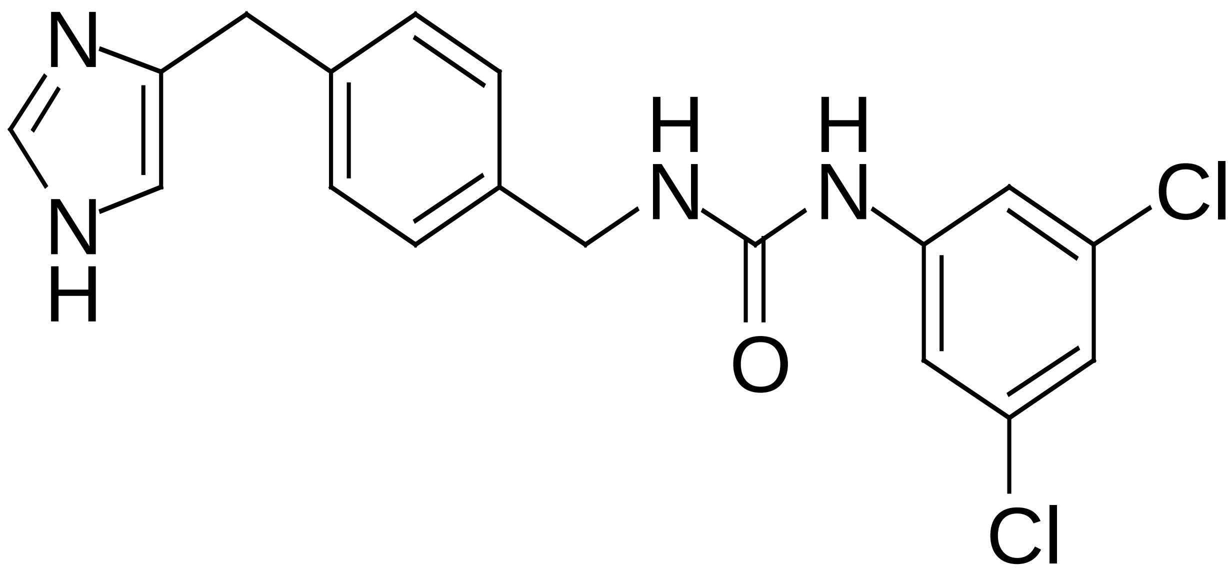

SCH 79687

Figure 1

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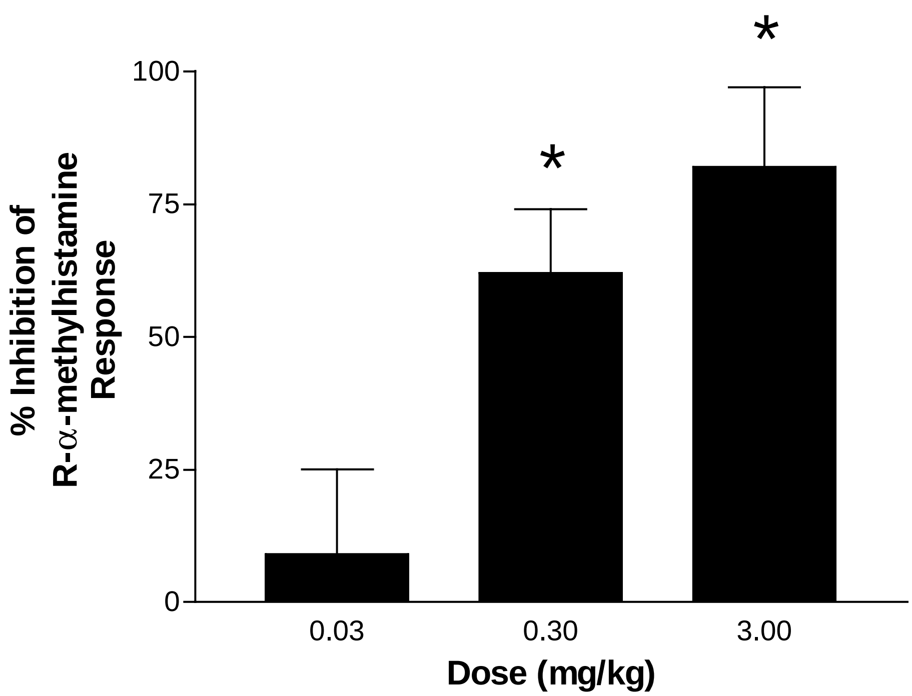

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Figure 2

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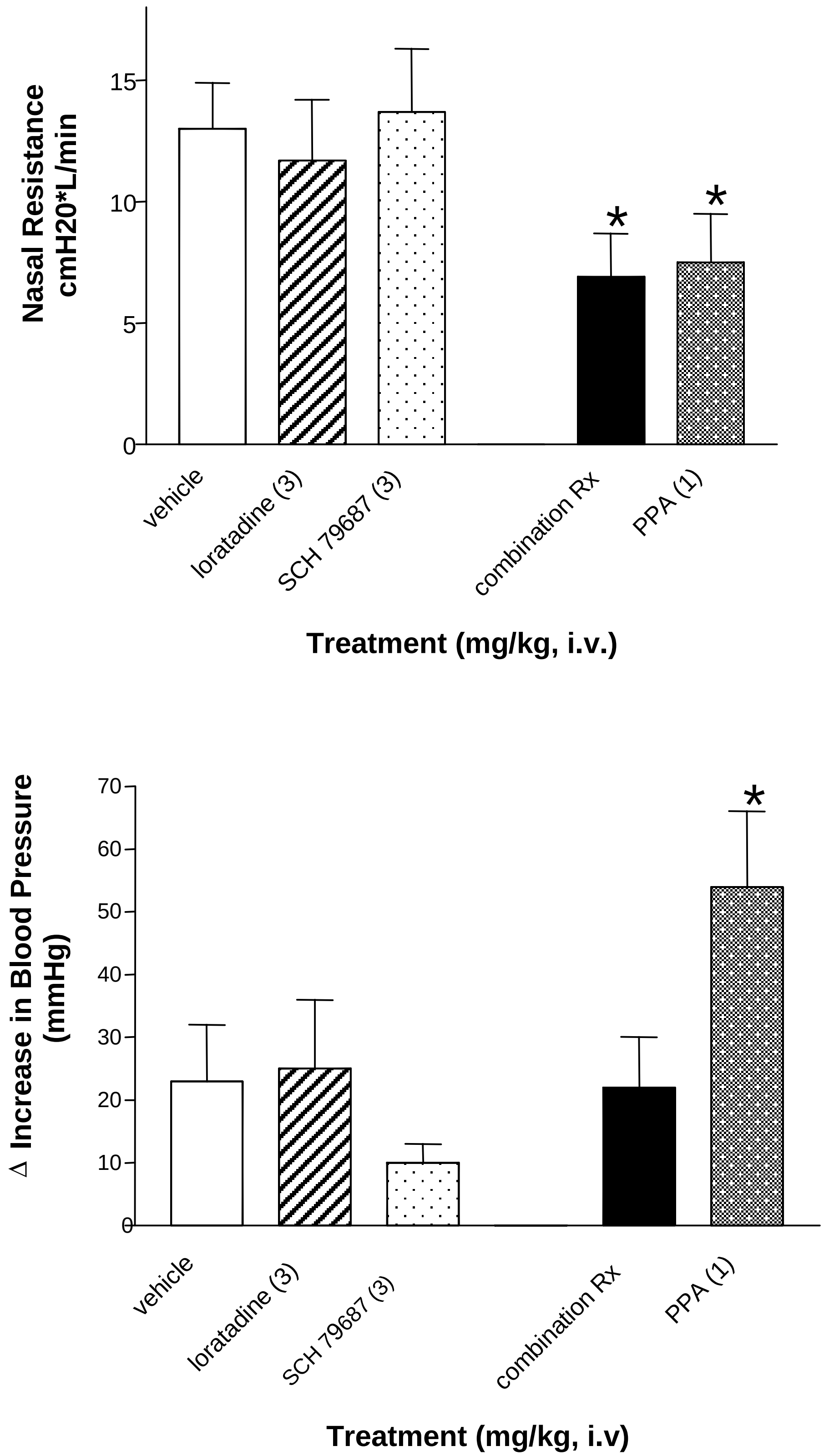

Figure 3

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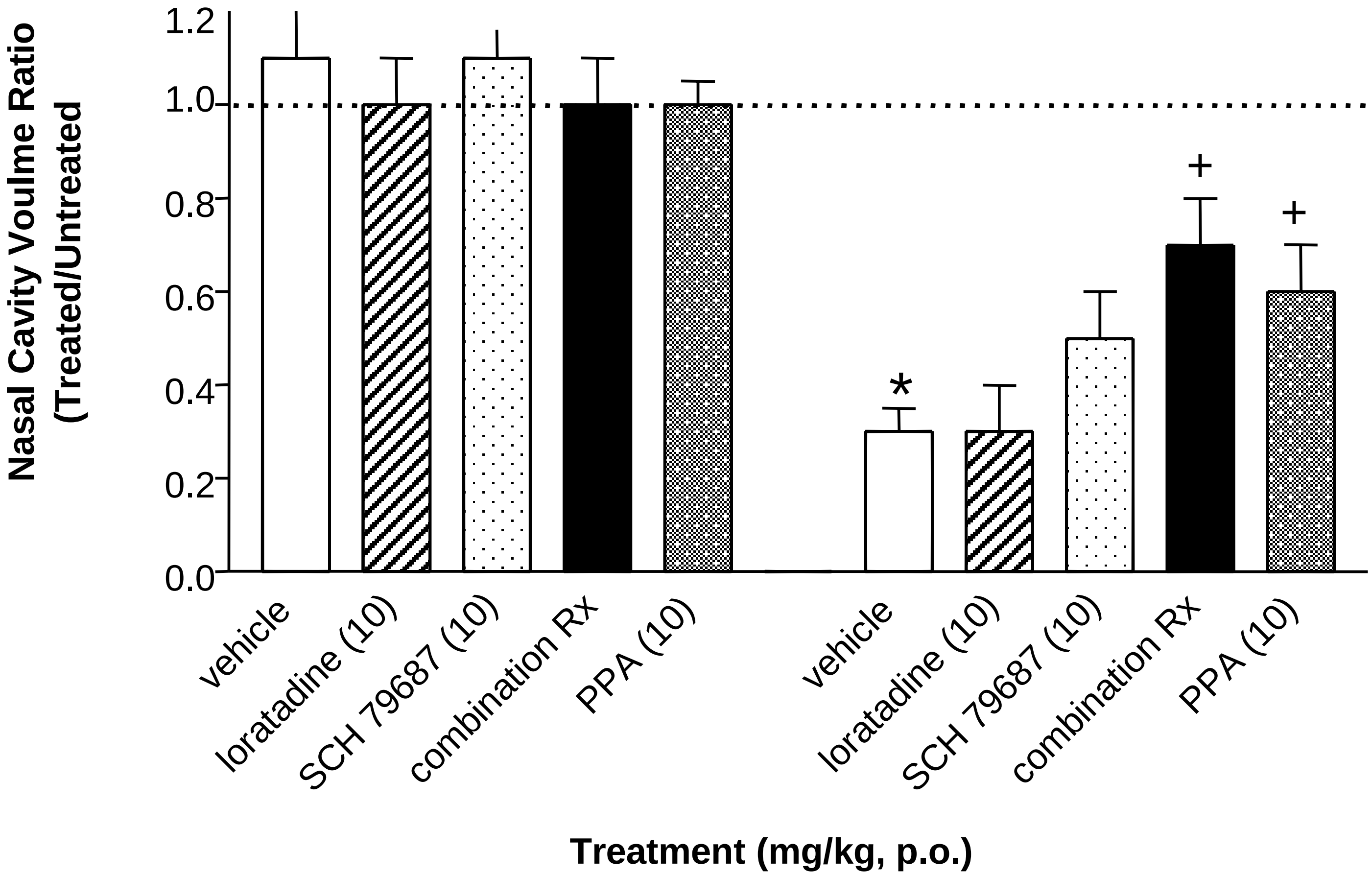

Figure 4

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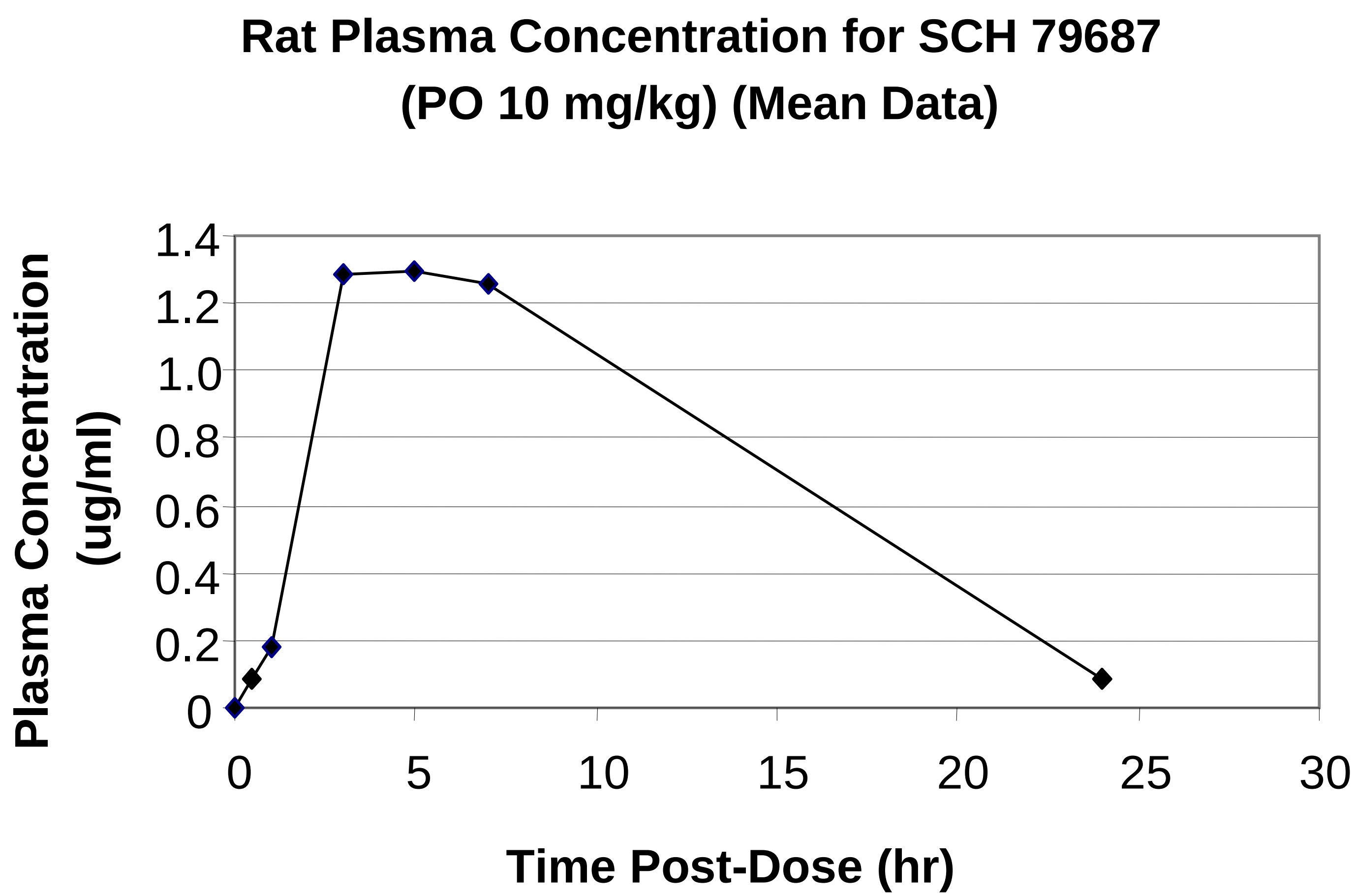

Figure 5

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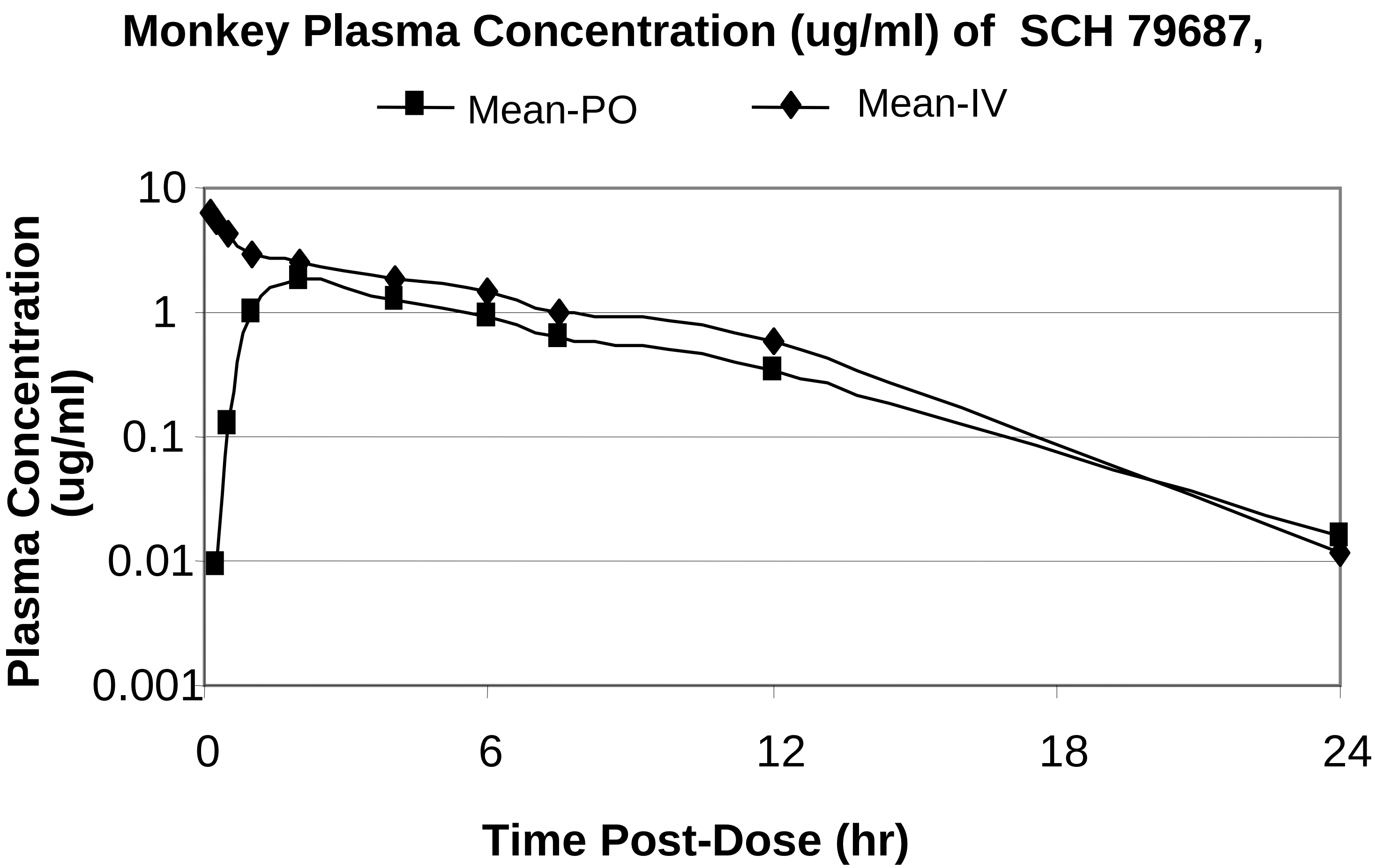

Figure 6

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