Pharmacology of \( N(3,5\text{-dichloro-1-oxido-4-pyridinyl})\-8\text{-methoxy-2-(trifluoromethyl)-5-quinoline Carboxamide (SCH 351591), a Novel, Orally Active Phosphodiesterase 4 Inhibitor}

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ABSTRACT

\( N(3,5\text{-dichloro-1-oxido-4-pyridinyl})\-8\text{-methoxy-2-(trifluoromethyl)-5-quinoline carboxamide (SCH 351591) has been identified as a potent (IC}_{50}/H_11005 58 \text{nM} and highly selective type 4 phosphodiesterase (PDE4) inhibitor with oral bioactivity in several animal models of lung inflammation. \( N(3,5\text{-dichloro-4-pyridinyl})\-8\text{-methoxy-2-(trifluoromethyl)-5-quinoline carboxamide (SCH 365351), the only significant in vivo metabolite, is also a potent and highly selective PDE4 inhibitor (IC}_{50} = 20 \text{nM}. Both SCH 351591 and SCH 365351 inhibited cytokine production in human blood mononuclear cell preparations. Oral SCH 351591 significantly attenuated allergen-induced eosinophilia and airway hyperreactivity in allergic guinea pigs at doses as low as 1 \text{mg/kg}. In this model, oral SCH 365351 showed similar potency. When SCH 351591 was administered orally to allergic cynomolgus monkeys at 3 \text{mg/kg}, \text{Ascaris suum} induced lung eosinophilia was blocked. Hyperventilation-induced bronchospasm in nonallergic guinea pigs, a model for exercise-induced asthma, was also suppressed significantly by oral SCH 351591 at 0.3 \text{mg/kg}. Cilomilast (SB 207499; Ariflo), a PDE4 inhibitor currently being developed for asthma and chronic obstructive pulmonary disease (COPD), was 10- to 30-fold less potent than SCH 351591 at inhibiting guinea pig lung eosinophilia and hyperventilation-induced bronchospasm. In a ferret model of emesis, maximum nonemetic oral doses of SCH 351591 and cilomilast were 5 and 1 \text{mg/kg}, respectively. Comparison of plasma levels at these nonemetic doses in ferrets to those at doses inhibiting hyperventilation-induced bronchospasm in guinea pigs gave a therapeutic ratio of 16 for SCH 351591 and 4 for cilomilast. Thus, SCH 351591 exhibits a promising preclinical profile as a treatment for asthma and COPD.

Asthma is a complex multifactorial disease characterized by reversible airway obstruction, airway inflammation, and nonspecific airway hyperreactivity (Mayer and Wills-Karp, 1999; Bertrand, 2000). Chronic obstructive pulmonary diseases (COPDs), on the other hand, are characterized by mostly irreversible airway obstruction due to chronic bronchitis and emphysema (Hay, 2000). Inflammation of the airways is believed to be central to the airways dysfunction in asthma and COPD (O'Shaughnessy et al., 1997; Roche, 1998). In these conditions, the airway wall is infiltrated by a variety of inflammatory cells, including mast cells, macrophages, T lymphocytes, eosinophils, and neutrophils. These cells release a host of mediators, including cytokines, chemokines, and bronchosplastic agents that act in concert with neurotransmitters such as acetylcholine and neurokinins from pulmonary nerves to produce bronchospasm, pulmonary edema, mucus hypersecretion, and other features of asthma and COPD. Eosinophilia is the dominant feature of lung inflammation in asthma, whereas COPD is marked by an intense pulmonary neutrophilia. Although bronchodilators such as \( \beta\)-agonists and anticholinergics are widely used for symptomatic relief,

**ABBREVIATIONS:** COPD, chronic obstructive pulmonary disease; PDE, cyclic nucleotide phosphodiesterase; SPA, scintillation proximity assay; DMSO, dimethyl sulfoxide; TNF\(_\alpha\), tumor necrosis factor-\( \alpha\); PBMC, peripheral blood mononuclear cell; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; PIP, pulmonary insufflation pressure; PD, provocative dose; BAL, bronchoalveolar lavage; MED, minimum effective dose; HIB, hyperventilation-induced bronchospasm; AUC, area under the curve; SCH 351591, \( N(3,5\text{-dichloro-1-oxido-4-pyridinyl})\-8\text{-methoxy-2-(trifluoromethyl)-5-quinoline carboxamide; SCH 365351, } N(3,5\text{-dichloro-4-pyridinyl})\-8\text{-methoxy-2-(trifluoromethyl)-5-quinoline carboxamide.}

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glucocorticoids are the only drugs currently available that effectively treat inflammation in asthma but not in COPD (Bertrand, 2000; Hay, 2000). One group of potential therapies for chronic pulmonary conditions is inhibitors of type 4 cAMP-specific phosphodiesterase (PDE4), of which theophylline, a nonspecific PDE inhibitor currently available for the treatment of asthma and COPD, is considered as prototypic (Barnette and Underwood, 2000).

There are at least 11 PDE enzyme families that degrade cAMP and/or cGMP (Torphy, 1998; Giembycz, 2000). In recent years, PDE4 has been widely pursued as a target to develop selective inhibitors with the hope of reducing the adverse effects associated with nonselective inhibitors such as theophylline. PDE4 is viewed as an exciting anti-inflammatory target for several reasons: 1) leukocyte functions are suppressed by cAMP, 2) PDE4 is the predominant isoform in inflammatory and immune cells, and 3) inhibitors of PDE4 negatively regulate the functions of almost all proinflammatory and immune cells and exert widespread anti-inflammatory activities in animal models of asthma. In recent years, selective PDE4 inhibitors have entered clinical trials, but most have failed due primarily to dose-limiting emesis and gastrointestinal disturbances (Martin, 2001). Colimilast and roflumilast represent a newer generation of PDE4 inhibitors (Barnette et al., 1998; Underwood et al., 1998; Torphy et al., 1999; Bundschuh et al., 2001; Hatzelmann and Schudt, 2001) and are now in advanced stages of clinical development, showing promising efficacy in allergic rhinitis, asthma, and COPD in phase II trials (Compton et al., 1999; Schmidt et al., 2001; Timmer et al., 2002).

In this article, we describe SCH 351591 (Fig. 1) as a novel, selective, potent PDE4 inhibitor. Oral efficacy of this compound was evaluated in several animal models of asthma, including inflammatory cell recruitment into the airways and airway hyperreactivity (two cardinal features of asthma) in allergic guinea pigs and cynomolgus monkeys, and hyper-ventilation-induced bronchospasm (a model of exercised-induced asthma) in guinea pigs. The emetic potential of SCH 351591 was evaluated in ferrets, a widely used model of emesis. We also present limited data on SCH 365351 (Fig. 1), the major, active metabolite of SCH 351591 found in mice, rats, and monkeys, which may contribute to the efficacy of SCH 351591 in these species. We conclude from these studies that SCH 351591 exhibits a biological profile predictive of its utility in pulmonary conditions such as asthma and COPD.

**Experimental Procedures**

**Materials.** SCH 351591, SCH 365351, and colimilast were synthesized at Celltech Chiroscience Ltd. (Cambridge, UK). Salbutamol was from Schering Plough (Kenilworth, NJ). [3H]cAMP, [3H]cGMP, [3H]rolipram, scintillation proximity assay (SPA) beads, and Ficoll-Paque were from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Rolipram was from RBI/Sigma (St. Louis, MO). Ascaris suum extract was from Greer Laboratories (Lenoir, North Carolina). Polyclonal human elevated IgE sera and affinity-purified polyclonal sheep anti-human IgE antibody were from The Binding Site (San Diego, CA). Heat-killed Bordatella pertussis was from Connaught Laboratories (North York, ON, Canada). PDE enzymes were obtained as described below. All other reagents were purchased from standard laboratory supply vendors including Sigma Chemical (St. Louis, MO) and Fisher Scientific (Springfield, NJ).

**PDE Enzyme Assays.** The PDE activity was determined radiometrically as described previously (Wang et al., 1997) by SPA. The assay mixture contained 50 mM Tris, pH 7.5, 8.3 mM MgCl2, 1.7 mM EGTA, various concentrations of inhibitor, and an aliquot of the enzyme solution in a final volume of 100 μl. After preincubation for 5 min at 30°C, the reaction was started by the addition of substrate (cAMP or cGMP). After incubation for an additional 30 min, the reaction was terminated by the addition of 900 μg of ytrrium silicate SPA beads, and the vials were counted for radioactivity. Sufficient enzyme was added to achieve 15 to 20% substrate breakdown.

Compounds were dissolved in dimethyl sulfoxide (DMSO) and serially diluted with Tris-HCl buffer described above to obtain the desired final concentration of the inhibitors at a DMSO concentration of 0.5% (v/v). This DMSO concentration affected none of the PDE activities. All data were analyzed using XlFit (a feature of Microsoft Excel) to construct inhibition curves and calculate IC50 values. For kinetic analysis, PDE4 activity at six different cAMP concentrations spanning Km (~5 μM) was measured in the absence of inhibitor. This was repeated in the presence of six different concentrations of inhibitor. Data were analyzed by reciprocal plots (1/v versus 1/s and 1/v versus s) to determine the type of inhibition.

Human PDE4 obtained from U937 cells by anion exchange chromatography was assayed in the presence of 0.125 μM cAMP and 20,000 cpm [3H]cAMP. Human PDE1 expressed in SF9 cells was assayed in the presence of 0.1 mM CaCl2, 125 U/ml calmodulin, 0.5 μM cAMP, and 20,000 cpm [3H]cAMP. PDE2 purified from human platelets by anion exchange chromatography was assayed in the presence of 0.5 μM cGMP, 0.5 μM cAMP, and 20,000 cpm [3H]cAMP. The PDE3 purified from human platelets by anion exchange chromatography was assayed in the presence of 0.125 μM cAMP and 20,000 cpm [3H]cAMP. PDE5 purified from human platelets by anion exchange chromatography was measured in the presence of 0.125 μM cGMP and 20,000 cpm [3H]cGMP. Human PDE7 purified from the HUT78 cell line by anion exchange chromatography was assayed in the presence of 0.034 μM cAMP and 20,000 cpm [3H]cAMP.

**Rolipram Binding Assay.** The rolipram binding assay was performed radiometrically in 96-well MAFC NOB filter plates (Millipore Corporation, Bedford, MA). The assay mixture contained 20 mM Tris-HCl, pH 7.5, 2 mM MgCl2, 0.1 mM dithiothreitol, 100 μg of rat brain (excluding the cerebellum) membrane proteins, various concentrations of compound, and 0.5% (v/v) DMSO. nonspecific binding was determined in the presence of 5 μM rolipram. The assay was started by the addition of 4 nM [3H]rolipram (10,000 cpm). After incubation at 22°C for 1 h, the reaction mixture was filtered, washed with 3 × 200 μl of ice-cold saline, the filters dried, 100 μl of scintillant added, and the plates left to stand for 1 h before counting for radioactivity.

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**SCH 351591**

**SCH 365351**

*Fig. 1. Structures of SCH 351591 and SCH 365351. SCH 365351, a deoxygenated metabolite of SCH 351591, was found in variable extents in mice, rats, and monkeys.*
Tumor Necrosis Factor-α (TNFα) Production by Peripheral Blood Mononuclear Cells (PBMCs). Human PBMCs were isolated fromuffy coats by centrifugation on a density gradient of Ficoll-Paque. PBMCs were harvested, washed three times, resuspended at 2 x 10⁶/ml in RPMI 1640 medium containing 2% fetal bovine serum, and 250-µl aliquots plated in 48-well tissue culture plates. Inhibitors were dissolved in DMSO, diluted in RPMI 1640 medium, and 100 µl added to duplicate wells at a range of concentrations (final DMSO concentration of 0.5%). Cells were stimulated with lipopolysaccharide (LPS) at a final concentration of 100 ng/ml and incubated for 18 h at 37°C in an atmosphere of 5% CO₂ and 95% air. Cells were pelleted by centrifugation, and TNFα was measured in the supernatant by ELISA (R & D Systems, Minneapolis, MN).

Interleukin (IL)-5 Production by Human Peripheral Blood Leukocytes. Inhibitors were dissolved in DMSO, diluted in RPMI 1640 medium, and 100 µl added to duplicate wells at a range of concentrations (final DMSO concentration of 0.5%). Buffcoat was diluted 2-fold with RPMI 1640 medium, and 200 µl was added to each well. Cells were stimulated by the addition of 100 µl of 10 µg/ml phytohemagglutinin to a final concentration of 2.5 µg/ml and 100 µl of 500 nM phorbol-12-myristate-13-acetate solution to a final concentration of 100 nM. The plate was then incubated for 48 h at 37°C in an atmosphere of 5% CO₂ and 95% air. Cells were pelleted, and IL-5 in the supernatant was measured by ELISA (R & D Systems).

IL-12 Production by Human PBMCs. Human PBMCs were isolated fromuffy coats by centrifugation on a density gradient of Ficoll-Paque. PBMCs were harvested, washed three times, resuspended at 3 x 10⁶/ml in serum-free RPMI 1640 medium, and 400-µl aliquots plated in 48-well tissue culture plates. Inhibitors were dissolved in DMSO, diluted in RPMI 1640 medium, and 100 µl added to duplicate wells at a range of concentrations (final DMSO concentration of 1%). Cells were stimulated with Pansorbin (Staphylococcus aureus suspension) at a final concentration of 0.075% and incubated for 18 h at 37°C in an atmosphere of 5% CO₂ and 95% air. Cells were pelleted by centrifugation, and IL-12 in the supernatant was measured by ELISA (R & D Systems).

Bronchodilator Activity in Isolated Guinea Pig Trachea. Cervical tracheal ring segments (5.0 mm in length) isolated from the Charles River Hartley strain of male guinea pigs (740–813 g) were equilibrated at 1.0 g of initial tension in Krebs' buffer (37°C) in the presence of indomethacin (2 µM) and contracted with histamine (10 µM). When the contraction stabilized (10–15 min), a cumulative concentration response with test compound (half-log concentration increments at 10-min intervals) was performed. The β-adrenoceptor agonist salbutamol (10 µM) was added at the end of each experiment to obtain a maximal reversal of the histamine-induced contraction. SCH 351591 and cilomilast stock solutions in DMSO were diluted with the assay buffer. Test compound-induced relaxations were normalized as percentage of maximum reversal.

Antiallergic Activity in Isolated Human Bronchus. Donor lung tissue was procured from five males and three females, 16 to 56 years old, by the International Institute for the Advancement of Medicine (Scranton, PA) and the Anatomic Gift Foundation (Woodbine, GA). Macroscopically normal lung tissue was procured after cancer resection from a 67-year-old female by the Cooperative Human Tissue Network (Eastern Division, University of Pennsylvania Medical Center, Philadelphia, PA). Hospital medications did not include corticosteroids, theophylline, or long-acting β-adrenergic agents. Lung tissue was received in physiological media on wet ice 20 to 48 h after removal. Bronchus segments (4–10-mm internal diameter) were isolated, prepared as 5-mm-wide epithelium-denuded transverse muscle strips, and used on the day of arrival.

Assays were performed in 37°C Krebs' buffer, pH 7.4, continuously aerated with 95% O₂, 5% CO₂ gas. Tissues were equilibrated at 1.0 g (<5-mm-diameter bronchus) or 2.0 g (>5-mm-diameter bronchus) initial tension, and a reference contraction to 30 µM carbocoll was obtained. Tissues were passively sensitized with 10% (v/v) human IgE sera in buffer (2-h incubation), washed twice, and then incubated 30 min with SCH 351591, aminophylline, or vehicle. Contraction was induced with anti-human IgE antibody (1:1000 dilution in buffer) and followed for 90 min. Anti-human IgE antibody-induced contractions were assessed as the increase of gram tension over baseline at 10-min intervals over a period of 90 min and normalized as percentage of carbachol-induced contraction.

Induction of Cytokines in Corynebacterium parvum-Primed Mice. For priming with C. parvum, BDF1 mice were injected intravenously with 0.5 mg of heat-killed cells of ATCC strain 11827. One week after priming, mice were challenged i.v. with LPS (20 µg/mouse). Serum cytokine levels were quantified by cytokine-specific ELISAs. The post-challenge levels of TNFα, IL-10, and IL-12 were measured in blood samples drawn at 1.5 h. Mice were dosed orally with either vehicle SCH 351591 2 h before the LPS challenge.

LPS-Induced Pulmonary Inflammation in Rats. Male Sprague-Dawley rats (250–300 g) were anesthetized by inhalation of isoflurane (flow rate 1 ml/min; supplemented with O₂). Using a Penn-Century microspray needle, 0.1 ml of a 100-µg/ml LPS solution in saline was injected into the trachea. Animals not challenged with the LPS solution received 0.1 ml of saline. Animals were placed on a heat pad until they recovered from anesthesia. Afterward, they were returned to their cages and allowed food and water ad libitum. All animals survived these manipulations and no additional interventions were required to ensure their survival. Animals fasted overnight were orally dosed with either cilomilast, SCH 351591, or vehicle (0.4% methylcellulose) 2 h before the LPS challenge.

At appropriate time points after intratracheal challenge with LPS, animals were surgically prepared with a tracheal cannula. Surgery was performed under anesthesia. The airways were flushed with 2 × 2 ml of 0.9% saline and the two washings pooled. Lavage fluid was centrifuged (350g, 4°C, 7 min), supernatant aspirated, erythrocytes lysed, and pellet washed in phosphate-buffered saline containing 10% heat-inactivated fetal calf serum and 10 µg/ml DNase I. The cell suspension was centrifuged, supernatant aspirated, and pellet resuspended in the same buffer. Total cell counts were performed using a Neubauer hemacytometer. Differential cell counts were conducted on Cytospin-prepared slides stained with Fisher's Leukostat stain. At least 200 cells were assessed per slide using standard morphological criteria to define mononuclear, neutrophilic, and eosinophilic cells.

Acute Allergic Bronchospasm in Guinea Pigs. Male Hartley guinea pigs were sensitized with an intraperitoneal injection (0.5 ml) of a saline suspension containing 100 mg/ml alum and 100 µg/ml ovalbumin. Additionally, each animal was primed with an intraperitoneal injection (0.3 ml) of heat-killed B. pertussis (20 optical units/ml). Animals were returned to their cages and allowed food and water ad libitum. After 27 days, animals were ready for use and were fasted overnight before study.

Animals were surgically prepared with a tracheal cannula under anesthesia induced with the combination of 87 mg/kg ketamine and 15 mg/kg xylazine given intramuscularly and were then mechanically ventilated using a rodent respirator at settings of 55 breaths/min with 3 ml of inflation per breath. A side-port pressure transducer linked to a chart recorder was used to measure changes in pulmonary insufflation pressure (PIP).

While maintained on the rodent respirator, animals were exposed (15 breaths) to the aerosol of either saline or 0.1% ovalbumin generated by an ultrasonic nebulizer (model 25; DeVilbiss, Somerset, PA) and measurements of PIP were conducted throughout the exposure to saline or ovalbumin. Baseline and peak bronchospasm were recorded and the percentage of increase in PIP was calculated from these values. Two hours before the challenge, animals were orally dosed with SCH 351591, theophylline, or vehicle (0.4% methylcellulose).

Guinea Pig Model of Allergic Airway Hyperreactivity and Pulmonary Inflammation. Male Hartley guinea pigs were sensitized exactly as described above. Animals fasted overnight before the
study were exposed to two antigen challenges, separated by 6 h, of either saline or 0.3% ovalbumin for 10 min each. The aerosol was generated by an ultrasonic nebulizer (model Ultra Neb99, DeVilbiss). To prevent anaphylactic bronchospasm, animals received the H\textsubscript{1} antagonist pyrilamine (10 mg/kg i.p.). Animals were returned to their cages and allowed food and water ad libitum.

Twenty-four hours after the first antigen challenge, animals were surgically prepared for PIP measurement as described above. Measurements of PIP were conducted before and during the intravenous administration of rising doses of acetylcholine (1, 3, 10, and 30 μg/kg). Acetylcholine was dissolved in saline and given in a volume of 1 ml/kg for each dose. Bronchospasm to acetylcholine was expressed as the percentage of change in PIP over baseline (%PIP). Values of %PIP were plotted against the dose of acetylcholine, and a provocative dose (PD) that caused a 100% increase in PIP (PD\textsubscript{100}) was calculated for each animal.

After the completion of acetylcholine treatment, the airways were flushed with 2 × 3 ml of 0.9% saline, and the two washings were pooled. Total cells and eosinophils in lavaged fluid were enumerated as described above for LPS-challenged rats.

Animals were orally dosed with either SCH 351591, SCH 365351, cilomilast, or vehicle (0.4% methylcellulose), 2 h before the first antigen challenge and then again 1 h before the second antigen challenge.

**Hyperventilation-Induced Bronchoconstriction in Guinea Pigs.** Studies were performed on male Hartley guinea pigs ranging in weight from 400 to 600 g. The animals were fasted overnight but given water ad libitum. Anesthesia was induced by intraperitoneal injection of 50 mg/kg sodium pentobarbital. Animals were prepared with tracheal, jugular venous, and esophageal catheters and were mechanically ventilated throughout the experiment with a rodent ventilator (Harvard Apparatus, Holliston, MA). The ventilation setting used for eupneic respiration was 1.25 ml/100 g at a frequency of 50 breaths/min.

Guinea pigs were placed in a whole-body plethysmograph and catheters connected to the outlet ports. A differential transducer measured the pressure difference across the wire mesh screen, which covered a 1-inch hole in the wall of the plethysmograph and was used to measure airflow. The airflow signal was integrated to a signal proportional to volume. Volume calibrations were performed with a 5-ml syringe. Transpulmonary pressure was measured with a differential pressure transducer (Validyne Engineering, Northridge, CA) as the pressure difference between the trachea and the esophagus. The volume, airflow, and transpulmonary pressure were monitored with a pulmonary analyzer (model 6; Buxco Electronics, Sharon, CT) and used for derivation of pulmonary resistance (R\textsubscript{L}) and dynamic lung compliance (C\textsubscript{DYN}). Both R\textsubscript{L} and C\textsubscript{DYN} were computed for each breath and digitally recorded every 6 s on a printer.

Hyperventilation was induced by increasing the respiratory rate from 50 to 185 breaths/min for 10 min. Tidal volume was not changed. After 10 min of hyperventilation, the respiratory rate was returned to the eupneic rates of 50 breaths/min. For the oral studies, the peak increase in R\textsubscript{L} over baseline due to hyperventilation was determined in animals receiving SCH 351591, aminophylline, cilomilast, or vehicle. All treatments were given 2 h before the hyperventilation challenge. In separate studies the ability of compounds to reverse the peak bronchospasm was also evaluated. For these reversal studies, animals were hyperventilated for 10 min, and 2 min later compounds were given i.v. The changes in R\textsubscript{L} over basal value were measured just before and then again 5 min after the i.v. administration of the compound, and these values were used to calculate percentage of reversal.

For the oral studies, SCH 351591, cilomilast, and aminophylline were given in a 0.4% methylcellulose suspension. For the i.v. studies, SCH 351591 was given in 100% DMSO (0.1 ml/animal), whereas salbutamol and aminophylline were dissolved in saline.

**Pulmonary Changes in Allergic Monkeys.** Twelve naturally allergic male monkeys (mean body weight 7.3 kg) were assigned to the study. On day 1 of the experiment, each animal was anesthetized with 10 mg/kg i.m ketamine, and anesthesia was maintained by continuous intravenous infusion of 0.05 to 0.15 mg/kg/min propofol. Animals were intubated with a cuffed endotracheal tube and intermittently positive pressure ventilation started with 100% oxygen. Blood pressure, body temperature, and arterial oxygen saturation were monitored.

Bronchial reactivity to histamine was then measured. Increasing doses of intravenous histamine dihydrochloride dissolved in saline (0.1–10 μg/kg) were given until a 100% increase in total respiratory resistance (measured using the forced oscillation technique) was obtained. Pulmonary mechanics returned to baseline between each dose of histamine. A rapid intravenous infusion of 10 ml/kg lactated Ringer’s solution was given, if necessary, toward the end of the histamine dosing to maintain arterial blood pressure. Next, fiber optic bronchoscopy was performed and a bronchoalveolar lavage (BAL) obtained by instilling and then withdrawing 2 × 10-ml aliquots of 0.9% saline into the right lung. BALs were kept on ice until processed. The plane of anesthesia was lightened and the monkeys were dosed via a stomach tube with 3 mg/kg SCH 351591 in 1 ml/kg methylcellulose vehicle followed by a 3-ml flush with vehicle, or an equal volume of vehicle alone.

Two hours later the monkeys were reanesthetized and the lungs were mechanically ventilated with 100% oxygen at 30 breaths/min with a tidal volume of 10 ml/kg to maintain end tidal CO\textsubscript{2} in the range 35 to 40 mm Hg. Pulmonary resistance and compliance were calculated from recordings of transpulmonary pressure and airflow. Once the mechanics measurements were stable, animals were given three vital capacity breaths, and baseline readings were taken. Each animal then inhaled 15 breaths of nebulized A. suum extract (Greer Laboratories) in saline at a concentration determined previously to give an acute allergic response [dilutions of 1:1–1:10,000 (v/v)]. Pulmonary mechanics were monitored for 5 min then the animals were recovered and returned to their cages.

Early in the morning of day 2 (time 24 h) each animal was anesthetized with ketamine, the histamine dose response was measured, and a BAL was performed on the left lung. After a 3-week rest period the treatments were crossed over and the experiment repeated until each animal received both treatments.

Any red blood cells present in the BAL samples were removed by lysis, and cell pellets were reconstituted in 0.9% saline. Total and differential cell counts were performed by manual methods using 200 cells for the differential count.

**Ferret Emesis Assay.** Male albino and fitch ferrets (0.9–1.3 kg; Eastwoods Directory Services, Godalming, Surrey, UK) were housed in groups of five per cage with free access to food and water. Compounds dissolved in syrup BP 1:1 in water were administered orally. The animals were then transferred to individual observation cages and observed continuously for a 4-h period. The behavior was recorded by videocamera, and the tapes were subsequently played back to assess emesis. Emesis was defined as rhythmic abdominal contractions that were either associated with the expulsion of the gastrointestinal contents (i.e., vomiting) or were not associated with the expulsion of the gastrointestinal contents (retching). Data are expressed as the number of animals that responded to the total number tested per dose. A dose response was constructed for each compound and at the maximum nonemetic dose, blood samples were drawn at various time points through the left jugular vein cannulae for the determination of compound concentration in the blood.

**Quantification of Compound Concentration in Blood.** Blood samples drawn from monkeys, guinea pigs, and ferrets were spun down, and the plasma was harvested and stored at −70°C. Aliquots of plasma samples (40 μl) were transferred into minivials, and 100 μl of acetonitrile containing 0.4 ng/μl of an internal standard was added. After vortexing and centrifugation, the supernatant was transferred to a high-performance liquid chromatography microfil.
Ailuquts (30 μl) of the supernatant were injected into a TSQ 7000 LC-APCI/MS/MS system equipped with an APCI source (Thermo Finnigan, San Jose, CA). The liquid chromatographic system included a 600 S controller, a 616 pump, and a 717 plus autosampler (Waters, Milford, MA). Chromatographic separation was achieved with a reverse phase liquid chromatography column (Luna 3 μm, phenyl-hexyl, 50 × 4.6 mm; Phenometex, Torrance, CA) using an acetonitrile/water gradient. Solvent A consisted of 20:80 acetonitrile/ water, with 0.6 ml of glacial acetic acid and 0.6 ml of 90% formic acid per liter of solvent. Solvent B consisted of 100% acetonitrile with 0.6 ml of glacial acetic acid and 0.6 ml of 90% formic acid. SCH 351591 (MH+ = 432) and SCH 365351 (MH+ = 416) were quantified using selected reaction monitoring; monitoring the product ions of m/z = 254 for both target compounds. The internal standard with MH+ = 398 was monitored by measuring the product ion of m/z = 378. Argon gas at 2.0 millitorr was used for collision-activated dissociation of the precursor ions. Dwell time for each precursor-product ion transition was 0.3 s. Standard curve samples containing both target compounds were run in duplicate with the same sets. The method was found to be linear from 5 to 5000 ng/ml. The limit of quantification was 5 ng/ml for both compounds.

Statistics. For in vivo studies in guinea pigs, rats, and mice, comparison between groups were performed using analysis of variance, and post hoc differences were assessed using Fisher’s protected least significant difference. This analysis was performed using StatView for Macintosh. Data from the monkey studies were analyzed by Student’s paired t test.

Animal Handling. All studies using animals were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act in a program approved by the American Association of the Accreditation of Laboratory Animals Care. Protocols used in these studies were approved by the Animal Care and Use Committee of Schering-Plough Research Institute.

Results

Inhibition of PDE4 and Rolipram-Binding Activity. Hydrolysis of [3H]cAMP to [3H]AMP by PDE4 from human monocytic U937 cells was monitored by scintillation proximity assay. The effect of PDE4 inhibitors on high-affinity rolipram binding was assessed by their ability to compete with binding of [3H]rolipram to rat brain membranes in a filtration binding assay. SCH 351591, a novel PDE4 inhibitor, showed higher affinity for PDE4 activity than either SCH 351591 or SCH 365351 at inhibiting high-affinity [3H]rolipram binding. SCH 365351, like cilomilast, inhibited LPS-induced TNFα, IL-5, and IL-12 production by human PBMCs in a dose-dependent manner (Table 2).

TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 PDE4</th>
<th>IC50 RBA</th>
</tr>
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<tbody>
<tr>
<td>SCH 351591</td>
<td>58 (42, 81)</td>
<td>153 (105, 223)</td>
</tr>
<tr>
<td>SCH 365351</td>
<td>20 (14, 27)</td>
<td>75 (49, 116)</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>86 (52, 140)</td>
<td>27 (16, 43)</td>
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</tbody>
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Fig. 2. Inhibition by SCH 351591 and aminophylline of anti-IgE antibody-induced contraction of isolated human bronchus. Contraction of muscle strips passively sensitized with human IgE sera was induced by anti-human IgE antibody. Contraction in the presence of vehicle, 1 μM SCH 351591, or 1 μM aminophylline was normalized to the 30 μM carbachol reference contraction measured before tissue sensitization, and the values are displayed as percent change from control. Contraction, as represented by 90-min AUC estimates, was significantly inhibited by SCH 351591 (p < 0.05, analysis of variance with Dunnett’s post hoc analysis).
2). SCH 351591 (IC_{50} = 59 nM) compared favorably with cilomilast (IC_{50} = 106 nM) but was less potent than SCH 365351 (IC_{50} = 12 nM). These compounds also inhibited IL-5 and IL-12 production by human blood leukocytes in a concentration-dependent manner (Table 2).

Anti-human IgE antibody induced contraction in passively sensitized isolated human bronchus. The peak contraction was significantly reduced (46%) by SCH 351591 at a concentration of 1 μM (Fig. 2). In this assay, 1 μM aminophylline was less effective than SCH 351591. Histamine-induced contraction of guinea pig trachea was potently reversed by the bronchodilator salbutamol (Fig. 3). In this assay, both SCH 351591 and cilomilast demonstrated poor activity, but their potencies were comparable with that of aminophylline (Fig. 3).

**Inhibition of LPS-Induced Cytokine Production of Cytokine in C. parvum-Primed Mice.** Mice previously primed with *C. parvum* produced TNFα, IL-10, and IL-12 after an intravenous administration of LPS as measured by increases in serum levels of these cytokines. Production of TNFα and IL-12 was inhibited by SCH 351591 in a dose-dependent manner with significant inhibition occurring at 2 mg/kg p.o. for TNFα and at 10 mg/kg p.o. for IL-12 (Table 3). In contrast, IL-10 production was enhanced by SCH 351591 treatment at all doses tested (Table 3), a finding consistent with the published observation that PDE4 inhibition enhanced the production of IL-10 in LPS-stimulated murine macrophages (Kambayashi et al., 1995).

**Inhibition of LPS-Induced Lung Neutrophilia in Rats.** Intratracheal administration of LPS to rats caused lung inflammation characterized by the appearance of neutrophils in the BAL fluid (Fig. 4). SCH 351591 at 3 mg/kg given orally 2 h before the LPS challenge inhibited neutrophil influx by 60%. No inhibition was seen at 0.3 mg/kg. In this model, cilomilast inhibited lung neutrophilia by 70% at

![Fig. 3. Reversal of histamine-induced contractions of isolated guinea pig tracheal rings. When the contraction of tracheal rings to histamine (10 μM) stabilized, a cumulative concentration response with each test compound was performed. Salbutamol (10 μM) was added at the end of each experiment to obtain the maximal reversal. Values are means ± S.E.M. of tissue responses normalized to the maximum reversal (n = 4–8 determinations).](image)

![Fig. 4. Inhibition by SCH 351591 and cilomilast of LPS-induced lung neutrophilia in rats. Sprague-Dawley male rats (5/group) were dosed orally with indicated doses of SCH 351591 or vehicle. Two hours later, mice were challenged i.v. with LPS (20 μg/mouse). Cytokine levels were measured by specific ELISAs in blood samples drawn 1.5 h after the challenge. Values are means ± S.E.M. (n = 8/group). Cytokines were not detectable in serum samples prepared from mice not treated with LPS. Numbers in parentheses represent percentage of inhibition.](image)

**TABLE 3**

<table>
<thead>
<tr>
<th>SCH 351591</th>
<th>Serum Cytokine</th>
<th>IL-10</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>TNFα</td>
<td>IL-12</td>
<td>IL-10</td>
</tr>
<tr>
<td>0</td>
<td>2300 ± 240</td>
<td>560 ± 50</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>1380 ± 140 (41)*</td>
<td>460 ± 30 (19)</td>
<td>12 ± 1*</td>
</tr>
<tr>
<td>10</td>
<td>540 ± 120 (77)*</td>
<td>300 ± 30 (47)*</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>20</td>
<td>480 ± 90 (79)*</td>
<td>260 ± 40 (56)*</td>
<td>19 ± 1*</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with LPS control.
TABLE 4

Effect of SCH 351591 on acute bronchospasm, airway reactivity, and lung inflammation in allergic guinea pigs

Two hours after a single dose of SCH 351591, sensitized guinea pigs (13–14/group) were challenged with OVA, and bronchospasm was measured and expressed as the percentage of the increase in PIP. A separate experiment, sensitized guinea pigs (19/group) were dosed orally with SCH 351591 or vehicle 2 h before the first antigen challenge and then again 1 h before the second antigen challenge. Twenty-four hours after the first challenge, PIP to intravenous ACh was measured and the PD of ACh that caused a 100% increase in PIP (PD100) was determined. After the completion of the acetylcholine experiment, BAL fluid was collected and analyzed for total cells and eosinophils. Numbers in parentheses represent the percentage of inhibition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SCH 351591</th>
<th>Airway Reactivity</th>
<th>BAL Cells × 10^6/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Airway Reactivity</td>
<td>PD100</td>
<td>Total Cells</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Increase in PIP %</td>
<td>μg ACh/kg</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>590 ± 60</td>
<td>11.9 ± 1</td>
</tr>
<tr>
<td>OVA</td>
<td>0.3</td>
<td>510 ± 50 (13)</td>
<td>11.2 ± 0.9a</td>
</tr>
<tr>
<td>OVA</td>
<td>3.0</td>
<td>340 ± 50 (42)a</td>
<td>14.6 ± 1.0a</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; N.D., not done; OVA, ovalbumin.

*p < 0.05 compared with OVA challenge.

Effects on Acute Bronchospasm, Airway Hyperreactivity, and Lung Inflammation in Allergic Guinea Pigs. Actively sensitized guinea pigs exposed to aerosolized ovalbumin developed an acute bronchospastic response. This response was significantly inhibited (71%) by theophylline (a nonselective PDE inhibitor) at a single oral dose of 100 mg/kg, given 2 h before the challenge (data not shown). When given orally, SCH 351591 inhibited (42%) bronchospasm significantly at 3 mg/kg (Table 4). However, in the same experiment, 21% inhibition observed at a higher dose (10 mg/kg) was not statistically significant (data not shown), suggesting that SCH 351591 exhibited only a partial efficacy in this model of acute bronchospasm.

When challenged with ovalbumin twice 6 h apart, actively sensitized guinea pigs developed a heightened responsiveness to acetylcholine as assessed by a reduced dose of acetylcholine for a fixed amount of response. PD100 (ie, dose of acetylcholine that increased PIP by 100%, was used as a measure of bronchial reactivity. PD100 decreased significantly (p < 0.05) in ovalbumin-treated animals compared with control animals (Tables 4–6), demonstrating development of airway hyperreactivity. This airway hyperreactivity was dose dependently blocked by SCH 351591 as evidenced by the inhibition of the decrease in PD100 (Table 4). Near complete inhibition of PD100 decrease was seen at 1 mg/kg p.o. SCH 351591, but a partial inhibition at 0.3 mg/kg was not statistically significant. SCH 365351 was also effective at inhibiting airway hyperreactivity, causing a complete inhibition of PD100 decrease at 1 mg/kg p.o. (Table 5). Interestingly, the PD100 value in ovalbumin-treated animals dosed with 10 mg/kg SCH 365351 was substantially higher than that in control animals (Table 5). A near complete inhibition of airway hyperreactivity by cilomilast was seen at 30 mg/kg p.o., whereas a partial inhibition at 3 mg/kg was not statistically significant (Table 6).

Sensitized animals challenged twice with antigen as described above also displayed a prominent pulmonary inflammation. This inflammation was characterized by an increase in the BAL recovery of total cells and eosinophils 24 h after the first antigen challenge (Tables 4–6). In this model, SCH 351591, given orally 2 h before the first antigen challenge and then again 1 h before the second antigen challenge, inhibited pulmonary recruitment of total cells and eosinophils in a dose-dependent manner with a minimum effective dose (MED) (defined as the minimum dose at which statistical significance is seen) of 1 mg/kg p.o. (Table 4). In a separate experiment not shown herein, the eosinophil influx in BAL fluid was inhibited by 88% at 10 mg/kg p.o. SCH 351591. SCH 365351 also was effective at inhibiting cellular influx into lungs (Table 5). In contrast, cilomilast at 30 mg/kg p.o. was ineffective at inhibiting cell migration into lungs (Table 5), although, in a separate experiment, a 30 mg/kg dose did cause a significant inhibition (51%) of total cell recruitment, but not of eosinophil recruitment. Thus, SCH 351591 is at least 30-fold more potent than cilomilast in this model.

Inhibition of Hyperventilation-Induced Bronchospasm (HIB) in Guinea Pigs. Given orally before the hyperventilation challenge, SCH 351591 dose dependently inhibited HIB with an MED of 0.3 mg/kg (Fig. 5A). SCH 351591 was about 10-times more potent than cilomilast (MED = 3 mg/kg p.o.). The nonselective PDE inhibitor aminophylline (30 mg/kg p.o.) caused an average inhibition of 43%, but this

TABLE 5

Effects of SCH 365351 on airway reactivity and lung inflammation in allergic guinea pigs

Airway reactivity and BAL cells were measured as described in Table 4. Numbers in parentheses represent the percentage of inhibition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SCH 365351</th>
<th>Airway Reactivity PD100</th>
<th>BAL Cells × 10^6/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>μg ACh/kg</td>
<td>Total Cells</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>12.7 ± 1.2</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>OVA</td>
<td>0</td>
<td>8.2 ± 1.1</td>
<td>5.4 ± 0.9</td>
</tr>
<tr>
<td>OVA</td>
<td>1</td>
<td>14.6 ± 1.7a</td>
<td>3.1 ± 0.7 (80)a</td>
</tr>
<tr>
<td>OVA</td>
<td>3</td>
<td>13.6 ± 1.4a</td>
<td>2.3 ± 0.6 (100)a</td>
</tr>
<tr>
<td>OVA</td>
<td>10</td>
<td>18.7 ± 2.2a</td>
<td>2.5 ± 0.4 (100)a</td>
</tr>
</tbody>
</table>

OVA, ovalbumin.

*p < 0.05 compared with OVA (n = 8 animals/group).
was not statistically significant (data not shown). Maximum inhibition attained by either SCH 351591 or cilomilast was 50 to 60%. The ability of intravenously administered SCH 351591 to reverse an ongoing HIB was evaluated in separate studies. Intravenous SCH 351591 reversed HIB in a dose-related manner with an MED of 0.3 mg/kg (Fig. 5B). Maximum reversal was 67% at 1 mg/kg. Intravenous aminophylline also produced a dose-related reversal of HIB (MED = 3 mg/kg), but was about 10-times less potent than SCH 351591. In contrast, the β-agonist salbutamol (MED = 0.001 mg/kg) was about 300-fold more potent than SCH 351591 at reversing HIB.

**Inhibition of Lung Inflammation in Allergic Monkeys.** When challenged with aerosolized *A. suum* extract, naturally allergic monkeys developed pulmonary inflammation characterized by an increased presence of total cells, eosinophils, and neutrophils in the bronchoalveolar lavage fluid collected 24 h after the antigen challenge (Table 7). When animals were given SCH 351591 at a single oral dose of 3 mg/kg 2 h before the antigen challenge, the influx of eosinophils in the bronchoalveolar lavage fluid was blocked by 80%. The influx of neutrophils was also inhibited. An acute bronchospasm induced by *A. suum* was unaffected by SCH 351591 (Table 7). The effect on bronchial hyperreactivity could not be ascertained because of large variability among animals (data not shown).

**Induction of Emesis in Ferrets.** When dosed orally with SCH 351591, one of eight ferrets retched at 8 mg/kg (Table 8). No emesis was seen at 5 mg/kg despite appreciable plasma levels ($C_{\text{max}} = 3.5 \mu g/ml$; AUC = 26.7 μg · h/ml). The maximal no-effect dose for SCH 365351 was 6 mg/kg. Cilomilast was emetic at 3 mg/kg, but not at 1 mg/kg. At 1 mg/kg, the $C_{\text{max}}$ and AUC values for cilomilast were 2.4 μg/ml and 18.3 μg · h/ml, respectively.

**Therapeutic Ratio for SCH 351591.** Studies in ferrets demonstrate that substantial plasma exposure of SCH 351591 and SCH 365351 can be achieved without emesis (see above). In the guinea pig efficacy model, SCH 351591 was active against hyperventilation-induced bronchospasm at an oral dose of 0.3 mg/kg with $C_{\text{max}}$ and AUC values of 0.24 μg/ml and 1.7 μg · h/ml, respectively (Table 9). In this model, the efficacious dose of cilomilast against hyperventilation-induced bronchospasm was 3 mg/kg ($C_{\text{max}} = 0.5 \mu g/ml$; AUC = 4.9 μg · h/ml). Comparison of the plasma levels of SCH 351591 at the guinea pig efficacy dose with those at the maximal no-effect dose of 5 mg/kg in ferrets ($C_{\text{max}} = 3.5 \mu g/ml$; AUC = 26.7 μg · h/ml) gives a therapeutic ratio of 16 for SCH 351591 (Table 9). A similar calculation gives a therapeutic ratio of 4 for cilomilast.

**Discussion**

Intensive effort over the last decade to develop PDE4 inhibitors for the treatment of lung inflammatory conditions such as asthma and COPD has yielded a number of potent and selective PDE4 inhibitors (Martin, 2001). Two of these inhibitors (GlaxoSmithKline’s cilomilast and Byk Gulden’s rolufilast) are currently in phase III, and several others are in various stages of clinical development. In this report, we describe SCH 351591 and its metabolite SCH 365351 as novel PDE4 inhibitors with in vitro potencies comparable with cilomilast. Published studies show that rolufilast is over 100-fold more potent than cilomilast at inhibiting PDE4 enzyme and in vitro TNFα production (Hatzelmann and Schudt, 2001). SCH 351591 and SCH 365351 did not discriminate between the four PDE4 (A, B, C, and D) subtypes, but they were highly selective versus other PDE isozymes. Importantly, in several animal models of asthma, SCH 351591 showed good efficacy (oral ED$_{50}$ of 0.3–1 mg/kg) against lung inflammation, bronchial hyperreactivity, and hyperventilation-induced bronchospasm. In these assays, SCH 351591 was 10- to 30-fold more potent than cilomilast. In guinea pig and rat models of lung function and inflammation, orally

### Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cilomilast</th>
<th>Airway Reactivity</th>
<th>BAL Cells $\times 10^6$/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>$\mu g$ ACh/kg</td>
<td>Cells</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>10.8 ± 1.9</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>OVA</td>
<td>0</td>
<td>5.4 ± 1.0</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>OVA</td>
<td>3</td>
<td>8 ± 1.8</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>OVA</td>
<td>30</td>
<td>9.9 ± 1.0</td>
<td>3.0 ± 0.4</td>
</tr>
</tbody>
</table>

OVA, ovalbumin. $^*$p < 0.05 compared with OVA (n = 6 animals/group).

---

Fig. 5. Modulation by various inhibitors of HIB in guinea pigs. HIB was induced by transiently increasing the respiratory rates from 50 to 185 breaths/min. Compounds at indicated doses were given either orally 2 h before the challenge to ascertain inhibition of bronchospasm (A) or intravenously 2 min after the challenge to ascertain reversal of bronchospasm (B). Bronchospasm was measured as pulmonary resistance ($R_L$) and the percentage of inhibition and the percentage of reversal of bronchospasm were calculated. Values are means ± S.E.M. (n = 6–12 animals/group). $^*$, $^*$p < 0.05 compared with vehicle control.
administered rolflumilast was shown to be 30- to 300-fold more potent than cilomilast (Bundschuh et al., 2001), suggesting that the in vivo profile of rolflumilast is likely to be superior to SCH 351591.

Compared by doses and by plasma levels of parent compound required for efficacy in guinea pigs and emesis in ferrets, SCH 351591 was 3- to 4-fold less emetic than cilomilast (Table 9). Several factors may contribute to the reduced emetic activity of SCH 351591. PDE4 exists in two unique conformations (Torphy et al., 1992, 1999; Torphy, 1998). One conformer binds rolipram with high affinity, and the occupation of these sites is associated with emesis and gastrointestinal disturbances. The other conformer binds rolipram much less avidly, and inhibition of this low-affinity form is correlated with anti-inflammatory actions. SCH 351591 and SCH 365351 bind to this high-affinity conformer with reduced avidity compared with rolipram. This reduced avidity of cilomilast for high-affinity rolipram sites (about 20-fold less than that of rolipram itself) has been offered as an explanation as to why cilomilast is less emetic than rolipram (Barnette et al., 1998; Torphy et al., 1999). Recent studies using gene-disrupted mice suggest that PDE4D is more involved in emesis than PDE4B (A. Robichaud, personal communication). Notably, cilomilast inhibits PDE4D with a 5- to 10-fold greater potency than it does PDE4B, whereas SCH 351591 does not distinguish between the two subtypes. Furthermore, our unpublished data showed that cilomilast (3 mg/kg p.o.) blocked gastrointestinal mobility in rats more drastically (96%) than SCH 351591 (48% at 6 mg/kg p.o.). Thus, the improved emetic profile of SCH 351591 may be the result of several factors, including equipotency against PDE4 subtypes, reduced avidity for high-affinity rolipram binding sites, and reduced potential for gastrointestinal disturbance.

SCH 351591 exhibited a poor bronchodilatory activity in isolated guinea pig trachea (Fig. 3). However, it was more effective at inhibiting allergen-induced contraction of passively sensitized human bronchus (Fig. 2). These data are consistent with the previously suggested view that the ability of PDE4 inhibitors to suppress antigen-induced contraction is due to the inhibition of mast cell degranulation rather than to direct bronchodilation (Underwood et al., 1993, 1998). Under in vivo as well as in vitro conditions, allergen-induced contraction of human airways results from the release of mediators such as histamine and cysteinyl leukotrienes (Heaslip et al., 1992). Lung mast cells contain PDE3 and PDE4 (Giembycz, 2000), and complete inhibition of antigen-driven mediator release from these cells and of contraction of passively sensitized human airways occurs only when both PDE3 and PDE4 are inhibited (Giembycz, 2000; Schmidt et al., 2000). It is important to note that SCH 351591 (3 mg/kg p.o.) did not block allergen-induced acute bronchospasm in our allergic monkeys (Table 7). It is possible that higher doses of the compound might be needed to block mast cell mediator release in these monkeys. The finding that SCH 351591 effectively blocked lung inflammation without affecting acute bronchospasm suggests that SCH 351591 is a more potent inhibitor of inflammation than of mast cell mediator release. Reversal of hyperventilation-induced bronchospasm in guinea pigs by intravenously administered SCH 351591 (Fig. 5B) suggests that, in this model where neu-

### TABLE 7

**Effects of SCH 351591 on A. suum-induced lung inflammation and acute bronchospasm in allergic monkeys**

This was a placebo-controlled crossover study involving 12 naturally allergic cynomolgus monkeys. SCH 351591 (3 mg/kg) or vehicle was given orally 2 h before the challenge with A. suum. Immediately after the challenge, lung resistance (RL) due to Ascaris was monitored for 5 min and expressed as percentage change over baseline. Twenty hours after the challenge, BAL fluid was collected and analyzed for total cells, eosinophils, and neutrophils. Values are expressed as means ± S.E.M. (n = 12). Numbers in the parentheses represent the percentage of inhibition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BAL Cells × 10⁶/ml</th>
<th>% change due to Ascaris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Cells</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Placebo</td>
<td>196 ± 41</td>
<td>27 ± 14</td>
</tr>
<tr>
<td>SCH 351591</td>
<td>168 ± 21</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>A. suum</td>
<td>646 ± 244</td>
<td>225 ± 62</td>
</tr>
<tr>
<td>Ascaris and SCH 351591</td>
<td>272 ± 31 (77)</td>
<td>50 ± 15 (79)</td>
</tr>
</tbody>
</table>

* p < 0.5 compared with A. suum treatment only.

### TABLE 8

**Emetic effects of SCH 351591, SCH 365351, and cilomilast in ferrets**

Ferrets were monitored for emetic episodes for 4 to 6 h after oral dosing.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Frequency</th>
<th>MND</th>
<th>Emesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH 351591</td>
<td>15</td>
<td>1/4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>SCH 351591</td>
<td>10</td>
<td>1/4</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>SCH 351591</td>
<td>8</td>
<td>1/8</td>
<td>5</td>
<td>0/8</td>
</tr>
<tr>
<td>SCH 365351</td>
<td>18</td>
<td>2/4</td>
<td>6</td>
<td>0/4</td>
</tr>
<tr>
<td>SCH 365351</td>
<td>15</td>
<td>1/8</td>
<td>6</td>
<td>0/4</td>
</tr>
<tr>
<td>SCH 365351</td>
<td>3</td>
<td>1/4</td>
<td>1</td>
<td>0/4</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>10</td>
<td>4/4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>3</td>
<td>1/4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>1</td>
<td>0/4</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

MND, maximal nonemetic dose.

### TABLE 9

**Therapeutic indices of SCH 351591 and cilomilast**

MED against hyperventilation-induced bronchospasm in guinea pigs (Fig. 5A) and maximum nonemetic dose (MND) against emesis in ferrets (Table 8) were determined under comparable experimental conditions for both compounds after a single dose.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCH 351591</th>
<th>Cilomilast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig HIB</td>
<td>MED (mg/kg p.o.)</td>
<td>0.3</td>
</tr>
<tr>
<td>Guinea pig HIB</td>
<td>C₁₀₀ (% MED)</td>
<td>0.24</td>
</tr>
<tr>
<td>Guinea pig HIB</td>
<td>AUC (µg·h/ml) @ MED</td>
<td>1.7</td>
</tr>
<tr>
<td>Ferret emesis</td>
<td>MND (mg/kg p.o.)</td>
<td>5</td>
</tr>
<tr>
<td>Ferret emesis</td>
<td>C₁₀₀ (% MND)</td>
<td>3.5</td>
</tr>
<tr>
<td>Ferret emesis</td>
<td>AUC (µg·h/ml) @ MND</td>
<td>26.7</td>
</tr>
<tr>
<td>Therapeutic ratio</td>
<td>C₁₀₀ @ MND/C₁₀₀ @ MED</td>
<td>15</td>
</tr>
<tr>
<td>Therapeutic ratio</td>
<td>AUC @ MND/AUC @ MED</td>
<td>16</td>
</tr>
</tbody>
</table>
neutrophils from the nerve endings are the major bronchospastic mediators. SCH 351591 could offer a significant bronchodilatory activity.

In recent clinical studies, orally administered cilomilast and roflumilast have been shown to improve lung function (forced expiratory volume in 1 s) significantly in patients with asthma and COPD (Compton et al., 1999, 2001; Timmer et al., 2002). This observation demonstrates that PDE4 inhibitors are capable of providing a clinically relevant level of bronchodilation in patients with asthma and COPD, despite the fact these compounds offer mild bronchodilatory activity in animal models. This beneficial effect on airflow may be attributed to the ability of cilomilast to attenuate bronchial hyperreactivity (Table 6) and hyperventilation-induced bronchospasm (Fig. 5A) and to amplify the cAMP-elevating effects of circulating catecholamines (Underwood et al., 1996). SCH 351591 was more potent than cilomilast against both bronchial hyperreactivity (Tables 4 and 6) and hyperventilation-induced bronchospasm (Fig. 5A) in our guinea pig models. Thus, SCH 351591 should compare favorably with cilomilast in its ability to improve forced expiratory volume in 1 s in patients with asthma and COPD.

It is believed that eosinophil inflammation mediated at least in part by the cytokine network is a major contributor to the characteristic nonspecific bronchial hyperreactivity in asthma (Venge, 1990). Like other PDE4 inhibitors (Torphy, 1998), SCH 351591 exerted marked inhibitory effects on the accumulation in the lungs of neutrophils and eosinophils in several species, including rats (Table 3), guinea pigs (Table 4), and monkeys (Table 7) in response to diverse stimuli (ovalbumin, LPS, and A. suum). In addition, SCH 351591 inhibited the production of proinflammatory cytokine TNFs, while enhancing the production of anti-inflammatory cytokine IL-10 (Tables 2 and 3). Thus, modulation of the cytokine network and inhibition of the eosinophil inflammation might have contributed to the effectiveness of SCH 351591 against airway hyperreactivity in allergic guinea pigs. Of note, however, in this regard is the fact that cilomilast inhibited bronchial hyperreactivity at doses where eosinophil infiltration remained unaffected (Table 6), suggesting that factors other than eosinophil might be involved. One such factor may well be the inhibition of the release of inflammatory neuropeptides such as substance P from the sensory nerve endings. This is suggested by our observation that hyperventilation-induced bronchospasm, a response believed to be mediated by the release of neuropeptides (Ray et al., 1989), was effectively attenuated by SCH 351591 and cilomilast (Fig. 5A). PDE4 inhibitors such as rolipram inhibit the excitatory nonadrenergic and noncholinergic neurotransmission (Undem et al., 1994) and augment the nonadrenergic and noncholinergic relaxation of bronchus (Fernandes et al., 1994), further supporting the potential impact of PDE4 inhibitors on neuronal control of pulmonary function. It is also possible that PDE 4 inhibitors affect aspects of airway remodeling such smooth muscle hypertrophy and goblet cell metaplasia, thereby contributing to their beneficial effect on airway hyperreactivity.

SCH 351591, like other PDE4 inhibitors, affected the trafficking of neutrophils into the lungs (Tables 3 and 7). Although neutrophils are generally recognized as part of the inflammatory process in COPD but not in asthma, recent data demonstrate pronounced neutrophilia in the lungs of asthma patients who are refractory to current treatments or suffer from severe exacerbations (Fahy et al., 1995; Wenzel et al., 1999; Norzila et al., 2000; Gibson et al., 2001). PDE4 inhibitors might prove beneficial in these patients.

In summary, we have identified SCH 351591 as a potent, selective, orally active PDE4 inhibitor. SCH 351591 blocked phosphodiesterase and bronchial hyperreactivity in animal models of asthma and COPD with excellent potencies. This pharmacological profile of SCH 351591 coupled with its mild bronchodilatory activity indicates its potential utility as an effective oral therapy for asthma and COPD.

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References


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