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SCH 48973: a Potent, Broad-Spectrum, Antienterovirus Compound

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SCH 48973 is a novel molecule with potent, selective, antienterovirus activity. In assays of the cytopathic effect against five picornaviruses, SCH 48973 had antiviral activity (50% inhibitory concentrations [IC_{50}s]) of 0.02 to 0.11 μg/ml, with no detectable cytotoxicity at 50 μg/ml. SCH 48973 inhibited 80% of 154 recent human enterovirus isolates at an IC_{50} of 0.9 μg/ml. The antiviral activity of SCH 48973 is derived from its specific interaction with viral capsid, as confirmed by competition binding studies. The affinity constant (K_i) for SCH 48973 binding to poliovirus was 8.85×10^{-8} M. In kinetic studies, a maximum of approximately 44 molecules of SCH 48973 were bound to poliovirus capsid. SCH 48973 demonstrated efficacy in a murine poliovirus model of enterovirus disease. SCH 48973 increased the survival of infected mice when it was administered orally at dosages of 3 to 20 mg/kg of body weight/day. Oral administration of SCH 48973 also reduced viral titers in the brains of infected mice. On the basis of its in vitro and in vivo profiles, SCH 48973 represents a potential candidate for therapeutic intervention against enterovirus infections.

The *Picornaviridae* family contains three groups of human pathogenic viruses: enteroviruses, rhinoviruses, and hepatitis A virus. These positive-strand RNA viruses share a common three-dimensional capsid structure which has been determined from the X-ray crystallization of human rhinovirus type 14 (HRV-14), HRV-1A, HRV-3, HRV-16, poliovirus type 1, poliovirus type 3, and coxsackievirus type B3 (13, 21, 25, 30, 32, 35, 40). The capsid, which encapsulates the viral RNA, is composed of 60 promoters, each of which contains one copy of the viral proteins VP1, VP2, VP3, and VP4. A significant feature on the viral capsid is the "canyon" formed by the junctions of VP1 and VP3. The canyon serves as the site for binding to the viral cellular receptor (e.g., ICAM-1 for HRV-14) (17, 42, 44). Underneath the canyon, within the VP1 protein, lies a hydrophobic cavity which is accessible through a pore in the canyon floor. The hydrophobic cavity is the drug-binding site of a number of diverse chemical structures that inhibit viral attachment and/or uncoating (2, 8, 11, 12, 14, 22, 24, 27, 33, 35, 37, 47). X-ray crystallography of drug-virus complexes confirms the specific binding of these inhibitors (4, 7, 49) and has allowed for the chemical synthetic design of newer, more potent compounds. Some capsid inhibitors have been investigated in clinical studies (1, 5, 18, 41, 46). However, to date none have been approved for use against diseases caused by rhinoviruses or enteroviruses.

The compound described here, SCH 48973, is a member of the SCH 47802 series of capsid-binding molecules (8). SCH 48973 is a potent, selective, antienterovirus molecule with in vitro and in vivo activities.

MATERIALS AND METHODS

Antipicornaviral molecules. SCH 48973 (Fig. 1) was synthesized at the Schering-Plough Research Institute (16).

Source of viruses. Enteroviruses representing the 15 most commonly isolated immunotypes in the United States were obtained from M. Pallansch, Centers for Disease Control and Prevention (CDC), Atlanta, Ga., or were collected by John Modlin, Darmouth Medical School. Laboratory strains of enteroviruses were obtained from the American Type Culture Collection, H. Rotbart (University of Colorado Health Sciences Center, Denver), and C. Gauntt (University of Texas Health Sciences Center, San Antonio). Poliovirus and coxsackievirus were propagated in HeLa cells, while RD and BGMK cells were used to propagate echoviruses and enteroviruses. The propagation of all viruses was done in Eagle's modified minimal essential medium (EMEM) with 1% fetal calf serum (FCS). Virus working stocks were prepared by standard procedures (39, 45).

CPE assay. The cytopathic effect (CPE) assay was used to determine the antiviral activity (50% inhibitory concentration [IC_{50}]), the cytotoxic activity (50% lethal concentration [LC_{50}]), and the therapeutic indices (cytotoxic LC_{50}/antiviral IC_{50}) of SCH 48973. The test compound and viruses were premixed for 45 min at 22°C and were used to infect cells (multiplicity of infection = 1.0) in 96-well tissue culture plates (Falcon 3075; Laboratory Disposable Products, North Haledon, N.J.). Following a 45-min incubation (37°C), the culture fluid was aspirated and the monolayers were washed. EMEM with 1% FCS containing the compound at the same concentration used for the preincubation step was added, and incubation was continued for 18 to 24 h prior to measuring cellular viability with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT formazan; Sigma Chemical Co.) (29). Uninfected cells were treated with compound in the same assay to determine the LC_{50}.

Plaque assay. A plaque reduction assay was used to evaluate the activity of SCH 48973 against a spectrum of viruses from the enterovirus family. The assay for coxsackieviruses was performed with HeLa cells, while RD cells were used for the majority of echoviruses and all enteroviruses. Some assays for echoviruses used BGMK cells. The assay was performed as described previously (8, 37). Briefly, 150 PFU of virus was mixed with test compound (0.0001 to 50 μg/ml in 1% dimethyl sulfoxide) in EMEM with 1% FCS for 45 min, and the mixture was added to monolayers of appropriate cells. After 45 min at 33°C, the inoculum and compound mixture were aspirated, and the cells were washed and incubated with a methyl cell overlay for 2 to 3 days without further addition of compound (37). The number of PFU was determined at each concentration and was plotted as a percentage of that for the control monolayers.

Radiolabelling of virus and viral purification. Virus was radiolabelled with [^{3}H]uridine and was purified by two consecutive rate zonal sedimentations in 30 to 70% sucrose gradients before pelleting (38).

Measurement of virus-bound SCH 48973. Purified poliovirus type 2 (2.47×10^{13} particles, as determined by optical density) was mixed with 1.150 pM [^{3}H]SCH 48973 (specific activity, 572.4 μCi/mg). It was assumed that SCH 48973 binds to noninfectious viral particles to the same extent as it binds to infectious viral particles (particle/PFU ratio, 235:1). The mixture was incubated at 22°C for prescribed times to allow SCH 48973 to bind to virus. Virus-bound and free SCH 48973 were separated by sedimentation in Sephadex G-50 NICK spin columns (fine grade; Pharmacia, Piscataway, N.J.) in TNE buffer (0.02 M Trizma, 0.5 M NaCl, 0.002 M EDTA [pH 7.2]) at $500 \times g$ for 5 min. The eluant was assayed for

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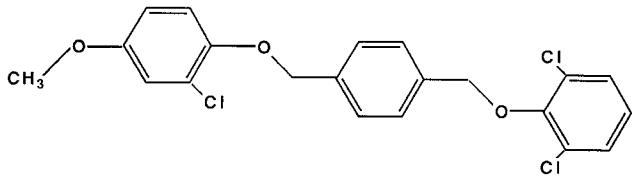

FIG. 1. Structure of SCH 48973.

radioactivity by liquid scintillation counting. The number of virion-bound SCH 48973 molecules was calculated by using the total disintegrations per minute recovered in the eluant and the specific activity of the [^{3}H]SCH 48973 preparation. In parallel studies, a 90% recovery of purified ^{3}H-labelled virions and PFU was observed and was used to correct for the loss of virus attributed to experimental procedures.

Determination of the affinity constant for SCH 48973 binding to virus. The affinity constant for SCH 48973 binding to poliovirus type 2 was determined by competition binding between [^{3}H]SCH 48973 and unradiolabelled SCH 48973. The approach assumed that binding is equivalent for infectious and noninfectious viral particles and that [^{3}H]SCH 48973 and unlabelled SCH 48973 possess equivalent binding affinities. The protocol used for competition binding was that described by Bennett (6). Briefly, 2.47×10^{13} particles of poliovirus type 2, 1.15 pmol of [^{3}H]SCH 48973, and concentrations of 10^{-1} to 10^{-9}M SCH 48973 were incubated for 3 h (22°C). TNE buffer (200 μl) was added, the contents were mixed, and the reaction mixtures were loaded onto a Sephadex G-50 NICK spin column to separate virion-bound and unbound SCH 48973. The eluant, which contained virus–[^{3}H]SCH 48973 complexes, was collected, and the radioactivity was directly counted. Results were plotted as the percentage of bound radioactivity versus the concentration of unlabelled SCH 48973.

Evaluation of SCH 48973 activity in animals. Studies with mice were performed as recommended elsewhere (30a). Groups of 15 to 25 male mice (weight, 16 to 20 g; Harlan Sprague-Dawley) were infected intracranially with poliovirus type 2 (23). Treatment with SCH 48973 was by oral gavage (0.3 ml in corn oil). Survival was monitored daily for 21 days. Results were evaluated for statistical significance by chi-square analysis (10), and protection was considered to be significant if the results met a probability of $p < 0.05$ for at least 5 days during the study compared to the results for the placebo-treated group.

Recovery of virus from murine brain. Mice were infected with poliovirus type 2 (520 PFU) and were treated (beginning either 6 or 24 h after infection) with SCH 48973 at 20 mg/kg of body weight/day (administered in four 5-mg/kg doses every 6 h). Forty-eight hours after infection, the brains were removed from euthanized mice and were frozen immediately in a methanol-dry ice bath. Tissues were weighed and homogenized for 1 min in dilution medium (EMEM with 1% FCS). After two cycles of freeze-thawing (methanol-dry ice bath), the tissues were centrifuged at $5,000 \times g$ for 10 min. The aqueous layer was removed from the pellet, and virus was quantitated by plaque assay.

RESULTS

CPE assay. The antiviral activity of SCH 48973 determined by an MTT-based CPE assay against five viruses of the enterovirus group demonstrates that SCH 48973 is a potent antiviral agent (Table 1). Against poliovirus type 2, coxsackievirus group A9, and three echovirus immunotypes, SCH 48973 has antiviral IC_{50}s ranging from 0.02 to 0.11 μg/ml. The lack of cytotoxicity at the highest concentration tested (50 μg/ml) indicates that the antiviral activity is not due to adverse effects on cells.

Spectrum of activity against enteroviruses. Surveillance data from the CDC indicate that 15 serotypes account for 65 to 89% of the enterovirus isolates recovered in a given year in the United States: echovirus types 3, 4, 5, 6, 7, 9, 11, 24, and 30; coxsackievirus group A9; and coxsackievirus groups B1, B2, B3, B4, and B5 (43). To determine the spectrum of activity of SCH 48973, 154 recent human isolates representing the 15 common enterovirus serotypes were tested by plaque reduction assay. Sixty-one isolates were collected by the CDC in the years 1990 to 1994. Ninety-three isolates were collected at Johns Hopkins University in the years 1986 to 1990.

The antiviral activities of SCH 48973 against the 154 isolates are presented in Fig. 2. SCH 48973 inhibited 80% of the immunotypes at an IC_{50} of 0.9 μg/ml. The median IC_{50} for 11 of 13 immunotypes was less than 1 μg/ml. Too few isolates of echovirus types 9 and 24 were tested to obtain an accurate representation of the median IC_{50}. The fact that SCH 48973 demonstrates potent inhibition against viruses plaqued on three different cell lines (i.e., HeLa, RD, and BGMK) indicates that the antiviral activity of the compound is not cell type dependent. Cytotoxicity assays with MTT endpoints, performed with HeLa, RD, and BGMK cells in a manner similar to that for the plaque assay, indicate that SCH 48973 possesses antiviral activity in the absence of significant cytotoxicity (data not shown).

Relation between enterovirus disease and antiviral activity. Of the 154 viruses representing the 15 most common immunotypes, 44 isolates were from undiagnosed individuals or patients with enterovirus diseases not associated with the central nervous system. The remaining viruses (71%; 110 of 154) were isolated from patients diagnosed with aseptic meningitis and/or meningoencephalitis. The average IC_{50} of SCH 48973 for these 110 viruses was 0.98 μg/ml. Ninety-three of these 110 isolates were collected at Johns Hopkins University in the years 1985 to 1990, and the site from which the virus was recovered was recorded. The site of recovery for the remaining 17 isolates was unknown. Of the 93 viruses from patients with aseptic meningitis and/or meningoencephalitis, 47% were from the cerebrospinal fluid, while the remaining viruses were from the throat, rectum, or urine. The historical frequency of enterovirus isolation in asymptomatic individuals is 3%, suggesting that viruses from non-central nervous system sites likely represent the causative agent of disease (27a).

Specificity. SCH 48973 exerts its antiviral effect exclusively against members of the picornavirus family. SCH 48973 is inactive against herpes simplex virus type 1 and type 2, influenza virus A, human immunodeficiency virus type 1, adenovirus type 5, human rotavirus type 1, respiratory syncytial virus, measles virus, Semliki Forest virus, simian virus 40, and vaccinia virus (data not shown).

Binding kinetics of SCH 48973. SCH 48973 was identified in an assay designed to detect compounds which stabilize poliovirus to heat inactivation. Consistent with its ability to stabilize poliovirus to heat inactivation, SCH 48973 is not virucidal (data not shown), a characteristic shared by other members of the SCH 47802 series (8). Data for other capsid inhibitors indicate that the ability to thermally stabilize picornaviruses is an independent but related measure of compound binding to viral capsid (3). To confirm the specific binding of SCH 48973 and to determine the number of molecules that interact with the capsid, the binding of [^{3}H]SCH 48973 to purified poliovirus type 2 particles was studied. The association of [^{3}H]SCH 48973 with viral particles was observed after the separation of free and bound molecules by sedimentation (Fig. 3). At maximum binding (3 h), 44.83 ± 5.08 molecules of SCH 48973 were

TABLE 1. Antiviral and cytotoxic activities of SCH 48973[a]

Virus	Antiviral activity (IC_{50} [μg/ml])	Cytotoxic activity (LC_{50} [μg/ml])
Poliovirus type 2	0.08 ± 0.04	>50.0
Coxsackievirus group A9	0.09 ± 0.07	>50.0
Echovirus type 4	0.11 ± 0.09	>50.0
Echovirus type 6	0.03 ± 0.005	>50.0
Echovirus type 11	0.02 ± 0.01	>50.0

[a] Data represent the means of at least four experiments and are expressed as the means and standard errors. HeLa cells were used for the analysis of poliovirus type 2 and coxsackievirus group A9. BGMK cells were used for the analysis of echoviruses. Poliovirus type 2 and coxsackievirus group A9 were from the American Type Culture Collection. Echoviruses were from M. Pallansch, CDC.

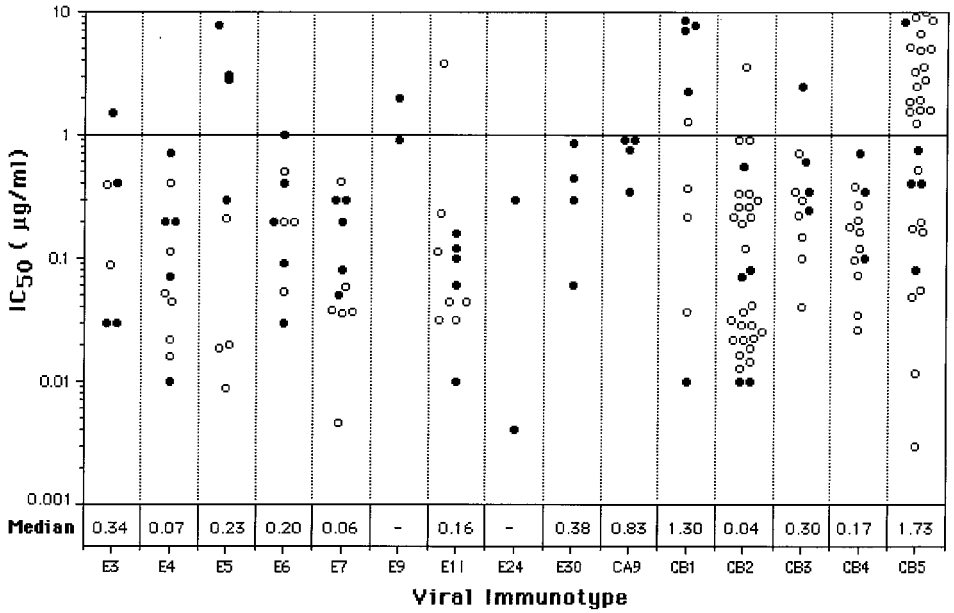

FIG. 2. Activity of SCH 48973 against recent human isolates of 15 immunotypes of enteroviruses. Open circles represent samples collected by the CDC, while closed circles represent samples collected at Johns Hopkins University.

bound per viral particle. On the basis of the number of VP1 hydrophobic pockets per viral capsid and assuming a ratio of one molecule per pocket, the theoretical maximum number of capsid-binding molecules that can bind is 60. These findings are consistent with the binding of SCH 48973 within the VP1 hydrophobic pocket.

Affinity of SCH 48973 for poliovirus type 2. The results of competition binding studies are presented in Fig. 4. The IC_{50}, which represents an estimation of the affinity constant (K_i) (6) for SCH 48973 binding to poliovirus type 2, was 8.85×10^{-8} M. Nonspecific binding of SCH 48973 to poliovirus type 2 particles was 3.79% and was determined from the binding of [^{3}H]SCH 48973 in the presence of a 2×10^6-fold excess of unlabelled SCH 48973 (Fig. 4).

Therapeutic efficacy of SCH 48973 in a poliovirus type 2 infection model. Figure 5 presents the results of efficacy studies with SCH 48973 at three dosages (3 to 20 mg/kg/day) in poliovirus type 2-infected mice. In these studies, SCH 48973 was administered therapeutically (3.5 h postinfection), and the total daily dosage was divided into three individual doses. SCH 48973 statistically increased survival at dosages of 3, 10, and 20 mg/kg/day compared to the survival rate for placebo-treated animals ($P \leq 0.05$). The degree of efficacy among dosages was not significantly different. Uninfected animals treated with SCH 48973 demonstrated no loss of weight and there was no mortality throughout this study or in other studies, including those in which SCH 48973 was administered at dosages of 90 mg/kg/day for 21 days (data not shown).

Reduction of virus in mouse brains by SCH 48973. For mice infected with poliovirus type 2 and treated (beginning either 6 or 24 h after infection) with SCH 48973 at 20 mg/kg/day, there was a significant reduction (1 to 2 logs) in the titer of virus recovered from the brains 2 days after infection (Table 2). The difference between the two regimens (6 and 24 h) was not significant. When brains from noninfected, SCH 48973-treated mice were removed, homogenized, diluted, and inoculated in vitro with known quantities of virus, no reduction of viral plaques compared to the input inoculum was observed (data not shown). These results indicate that the reduction in viral load in brains is due to a direct effect of SCH 48973 in vivo and not due to compound carryover into the tissue culture system.

DISCUSSION

SCH 48973 has potent in vitro activity against viruses of the enterovirus family, as demonstrated in CPE and plaque reduction assays. Antiviral testing against viruses of the 15 most

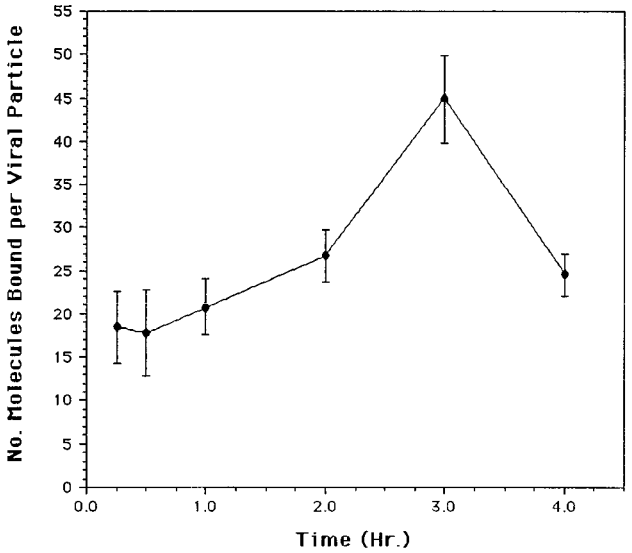

FIG. 3. Binding kinetics of SCH 48973 to poliovirus type 2. [^{3}H]SCH 48973 was allowed to bind to poliovirus particles at 22°C for the indicated times prior to separating bound and free [^{3}H]SCH 48973. The results represent means and standard errors of two studies, each with three replicate samples per time point. A decrease in the optical density at 280 nm was observed at the 4-h time point, suggesting that the decrease in the number of bound molecules was due to precipitation of viral particles.

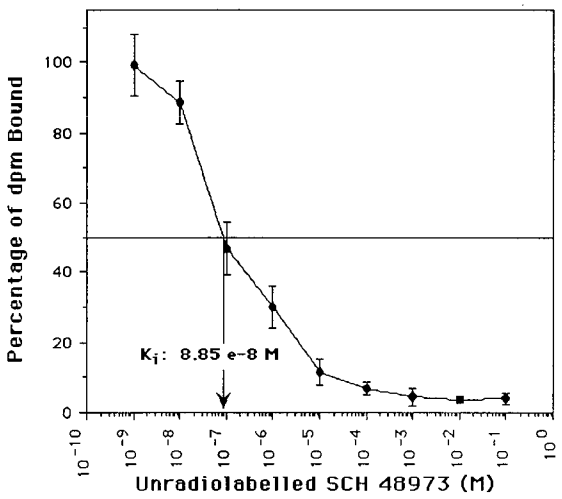

FIG. 4. The K_i for SCH 48973 binding to poliovirus type 2 was determined in competition binding studies with [^{3}H]SCH 48973. Incubation was performed until equilibrium was reached (3 h at 22°C). The results represent means ± standard errors of two different studies, each with three replicates per concentration.

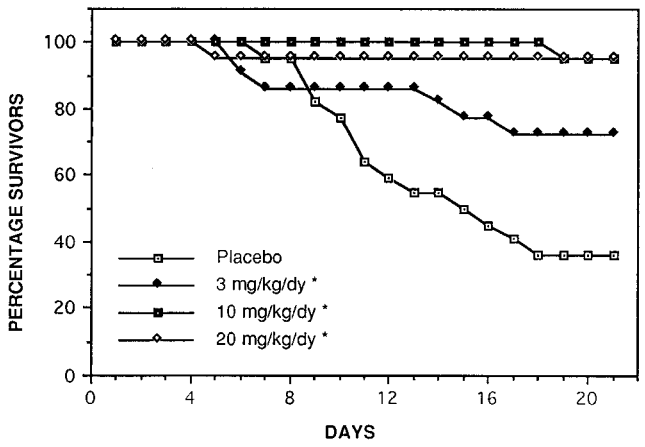

FIG. 5. Efficacy of SCH 48973 in poliovirus type 2-infected mice. Treatment was initiated 3.5 h after infection and was continued for 21 days. SCH 48973 was administered three times a day to achieve the total daily dose indicated. An asterisk indicates significance compared to the placebo group at a P value of ≤0.05 by the chi-square test for ≥5 days during the study.

commonly isolated enterovirus serotypes indicates that SCH 48973 inhibits 80% of the immunotypes (154 viruses) at an IC_{50} of 0.9 μg/ml, a concentration that is within the levels of the molecule achievable in plasma after oral dosing in higher animals (data not shown). SCH 48973 demonstrates similar activity against other enteroviruses which are not common in the United States but which are widespread throughout the world. For example, the concentration of SCH 48973 showing activity against five strains of poliovirus type 1 (causative agent of poliomyelitis) is 0.02 μg/ml and that against four strains of enterovirus 70 (causative agent of acute hemorrhagic conjunctivitis) (19, 20) is 0.02 μg/ml (data not shown). The basis for this broad-spectrum activity is (i) that the structural organization of the viral capsid and VP1 drug-binding pocket is shared by different serotypes and (ii) that SCH 48973 has high specific affinity for the viral capsid.

Binding kinetics and competition binding studies both support the specific interaction of SCH 48973. The number of SCH 48973 molecules which bind to the viral capsid (44.83 ± 5.08) is consistent with the results of other investigators (4, 15), who demonstrated 40 to 60 molecules of representative inhibitors from the WIN series to be bound to human rhinovirus type 14. The affinity constant of SCH 48973 is also within the values described for inhibitors from the WIN series bound to human rhinovirus type 14. Mechanistic studies (data not shown) indicate that SCH 48973 is inhibitory early in the virus replication cycle at the steps of viral attachment and/or uncoating, as described for a previous molecule in the series, SCH 47802 (8). Through interactions with specific amino acids within the VP1 cavity, capsid-binding inhibitors cause conformational changes within the VP1 cavity, the canyon (the site of virus cell receptor interaction with the drug), and protein loops which lie outside the canyon structure (34, 50). Comparative studies of the perturbations caused by SCH 38057 and WIN 51711 indicate that the number and extent of conformational changes do not correlate with antiviral activity (50). One current hypothesis is that the degree of "space filling" within the drug-binding pocket is critical for potent antiviral activity (31, 50). The specific interactions of SCH 48973 within the drug-binding pocket will be deciphered since the cocrystallization, X-ray diffraction, and spatial resolution of SCH 48973 in a picornavirus have recently been accomplished (3a).

The potential activity of SCH 48973 against picornavirus disease was determined by using poliovirus type 2-infected mice. This model is a stringent evaluation of antiviral efficacy, since direct intracranial inoculation of poliovirus leads to high viral titers in target tissues, resulting in paralysis and mortality. SCH 48973 orally administered at therapeutic levels significantly increased the survival rate for infected mice at dosages as low as 3 mg/kg/day (1 mg/kg given three times a day). The potency of SCH 48973 was also demonstrated by the reduction of viral titers in the brain after therapeutic dosing. In studies in which dosing was stopped on day 21, mice continued to survive for an observation period of at least 6 months, indicating that viral infection was halted in these animals (data not shown). The overall in vivo efficacy of SCH 48973 after oral administration indicates that the compound has sufficient pharmacokinetic properties to generate inhibitory levels at sites of virus replication.

The in vitro and in vivo profiles of SCH 48973 suggest that it is a potential candidate for the treatment of enterovirus infections. To date there is no approved antiviral agent for the treatment of enterovirus infections. While respiratory illness has been the focus of the clinical efforts in developing antipicornavirus molecules, illnesses such as aseptic meningitis (28, 36), febrile illness (9), persistent infections in agammaglobulinemic patients (26), and acute hemorrhagic conjunctivitis (20, 48) are also potential targets for therapy.

TABLE 2. Reduction of viral titers in mouse brains after oral administration of SCH 48973

Regimen[a]	Log_{10} PFU
Placebo	5.6
Four times, 5 mg/kg (6 h)	4.4[b]
Four times, 5 mg/kg (24 h)	3.3[b]

[a] Mice were infected with poliovirus type 2 (520 PFU). SCH 48973 was orally administered four times at 5 mg/kg (20 mg/kg/day) beginning either 6 or 24 h after dosing. Brains were removed on day 2 after infection, and the amount of virus was measured by plaque assay.

[b] Statistically significant efficacy compared to placebo group by Student's t test ($P < 0.05$).

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REFERENCES

1. **Al-Nakib, W., P. G. Higgins, G. I. Barrow, D. A. J. Tyrrell, K. Andries, G. Vanden Bussche, N. Taylor, and P. A. J. Janssen.** 1989. Suppression of colds in human volunteers challenged with rhinovirus by a new synthetic drug (R61837). Antimicrob. Agents Chemother. **33:**522–525.
2. **Andries, K., B. Dewindt, M. De Brabander, R. Stokborekx, and P. A. J. Janssen.** 1988. *In vitro* activity of R61837, a new rhinovirus compound. Arch. Virol. **101:**155–167.
3. **Andries, K., B. Dewindt, J. Snoeks, and R. Willebrords.** 1989. Lack of quantitative correlation between inhibition of replication of rhinoviruses by an antiviral drug and their stabilization. Virology **106:**51–61.

3a. **Arnold, E. J.** Personal communication.

4. **Badger, J., I. Minor, M. J. Kremer, M. A. Oliveira, T. J. Smith, J. P. Griffith, D. M. A. Guerin, S. Krishnaswamy, M. Luo, M. G. Rossmann, M. A. McKinlay, G. D. Diana, F. J. Dutko, M. Fancher, R. R. Rueckert, and B. A. Heinz.** 1988. Structural analysis of a series of antiviral agents complexed with human rhinovirus 14. Proc. Natl. Acad. Sci. USA **85:**3304–3308.
5. **Barrow, G. I., P. G. Higgins, D. A. J. Tyrrell, and K. Andries.** 1990. An appraisal of the efficacy of the antiviral R61837 in rhinovirus infections in human volunteers. Antivir. Chem. Chemother. **5:**279–283.
6. **Bennett, J. P.** 1978. Methods in binding studies, p. 57–90. *In* H. I. Yamamura, S. J. Enna, and M. J. Kuhar (ed.), Neurotransmitter receptor binding. Raven Press, New York, N.Y.
7. **Chapman, M. S., I. Minor, M. G. Rossmann, G. D. Diana, and K. Andries.** 1991. Human rhinovirus 14 complexed with antiviral compound R 61837. J. Mol. Biol. **217:**455–463.
8. **Cox, S., P. J. Buontempo, J. Wright-Minogue, J. L. DeMartino, A. M. Skelton, E. Ferrari, J. Schwartz, E. J. Rozhon, C. C. Linn, V. Girijavallabhan, and J. F. O'Connell.** 1996. Antipicornavirus activity of SCH 47802 and analogues: *in vitro* and *in vivo* studies. Antivir. Res. **32:**71–79.
9. **Dagan, R., C. B. Hall, K. R. Powell, and M. A. Menegus.** 1989. Epidemiology and laboratory diagnosis of infection with viral and bacterial pathogens in infants hospitalized for suspected sepsis. J. Pediatr. **115:**351–356.
10. **Davies, O. L., and P. L. Goldsmith.** 1972. Statistical methods in research and production, p. 317–323. Hasfner Publishing Company, New York, N.Y.
11. **Diana, G. D., M. A. McKinlay, C. J. Brisson, E. S. Zalay, J. V. Miralles, and U. J. Salvador.** 1985. Isoxazoles with antipicornavirus activity. J. Med. Chem. **28:**748–752.
12. **Eggers, H. J., M. A. Koch, A. Furst, G. D. Daves, Jr., J. J. Wilczynski, and K. Folkers.** 1970. Rhodanine: a selective inhibitor of the multiplication of echovirus 12. Science **167:**294–297.
13. **Filman, D. J., R. Syed, M. Chow, A. J. Macadam, P. D. Minor, and J. M. Hogle.** 1989. Structural factors that control conformational transitions and serotype specificity in type 3 poliovirus. EMBO J. **8:**1557–1579.
14. **Fox, M. P., M. J. Otto, and M. A. McKinlay.** 1986. Prevention of rhinovirus and poliovirus uncoating by WIN 51711, a new antiviral drug. Antimicrob. Agents Chemother. **30:**110–116.
15. **Fox, M. P., M. A. McKinlay, G. D. Diana, and F. J. Dutko.** 1991. Binding affinities of structurally related human rhinovirus capsid-binding compounds are related to their activities against human rhinovirus type 14. Antimicrob. Agents Chemother. **35:**1040–1047.
16. **Girijavallabhan, V., A. Ganguly, and R. Versace.** September 1995. U.S. patent 5,350,772.
17. **Greve, J. M., G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, and A. McClelland.** 1989. The major human rhinovirus receptor is ICAM-1. Cell **56:**839–847.
18. **Hayden, F. G., A. Andries, and P. A. J. Janssen.** 1992. Safety and efficacy of intranasal pirodavir (R77975) in experimental rhinovirus infection. Antimicrob. Agents Chemother. **36:**727–732.
19. **Hierholzer, J. C., K. A. Hilliard, and J. J. Esposito.** 1975. Serosurvey for "acute hemorrhagic conjunctivitis" virus (enterovirus 70) antibodies in the southeastern United States, with review of the literature and some epidemiologic implications. Am. J. Epidemiol. **102:**533–544.
20. **Higgins, P. G., P. J. Scott, P. M. Daniels, and D. R. Gamble.** 1974. A comparative study of viruses associated with acute hemorrhagic conjunctivitis. J. Clin. Pathol. **27:**292–296.
21. **Hogle, J. M., M. Chow, and D. J. Filman.** 1985. Three-dimensional structure of poliovirus at 2.9 Å resolution. Science **229:**1358–1365.
22. **Ishitsuka, H., C. Ohsawa, T. Ohiwa, I. Umeda, and Y. Suhara.** 1982. Antipicornavirus flavone Ro 09-0179. Antimicrob. Agents Chemother. **22:**611–616.
23. **Jubelt, B., G. Ghislaine, O. Narayan, and R. T. Johnson.** 1980. Pathogenesis of human poliovirus infection in mice. 1. Clinical and pathogenesis studies. J. Neuropathol. Exp. Neurol. **39:**138–148.
24. **Kenny, M. T., J. K. Dulworth, T. M. Bargar, and J. K. Daniel.** 1987. Antipicornavirus activity of some diaryl methanes and aralkylamino-pyridines. Antivir. Res. **7:**87–97.
25. **Kim, S., T. J. Smith, M. S. Chapman, M. G. Rossmann, D. Pevear, F. J. Dutko, J. P. Felock, G. D. Diana, and M. A. McKinlay.** 1989. Crystal structure of human rhinovirus serotype 1A (HRV 1A). J. Mol. Biol. **210:**91–111.
26. **McKinney, R. I., S. L. Katz, and C. M. Wilfert.** 1987. Chronic enteroviral meningoencephalitis in agammaglobulin patients. Rev. Infect. Dis. **9:**334–356.
27. **McSharry, J. J., L. A. Caliguiri, and H. J. Eggers.** 1979. Inhibition of uncoating of poliovirus by arildone, a new antiviral drug. Virology **97:**307–315.

27a. **Modlin, J.** Personal communication.

28. **Modlin, J. F.** 1990. Coxsackieviruses, echoviruses, and newer enteroviruses, p. 1367–1383. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious disease, 3rd ed. Churchill Livingstone, Inc., New York, N.Y.
29. **Mossmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65:**55–63.
30. **Muckelbvauer, J. K., M. Kremer, I. Minor, G. Diana, F. Dutko, J. Groake, D. C. Pevear, and M. G. Rossmann.** 1995. The structure of coxsackievirus B3 at 3.5 Å resolution. Structure **3:**653–667.

30a. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals. NIH publication 85-23. National Institutes of Health, Bethesda, Md.

31. **O'Connell, J. F., R. Albin, D. Blum, P. Grint, and J. Schwartz.** 1995. Development of antiviral agents for picornavirus infections, p. 419–434. *In* H. A. Rotbart (ed.), Human enterovirus infections. ASM Press, Washington, D.C.
32. **Oliveira, M. A., R. Zhao, W.-M. Lee, M. J. Kremer, I. Minor, R. R. Rueckert, G. D. Diana, D. C. Pevear, F. J. Dutko, M. A. McKinlay, and M. G. Rossmann.** 1993. The structure of human rhinovirus 16. Structure **1:**51–68.
33. **Otto, M. J., M. P. Fox, M. J. Fancher, M. F. Kuhrt, G. D. Diana, and M. A. McKinlay.** 1985. In vitro activity of WIN 51711, a new broad-spectrum antipicornavirus drug. Antimicrob. Agents Chemother. **27:**883–886.
34. **Pevear, D. C., M. J. Fancher, P. J. Felock, M. G. Rossmann, M. S. Miller, G. Diana, A. M. Treasurywala, M. A. McKinlay, and F. J. Dutko.** 1989. Conformational change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors. J. Virol. **63:**2002–2007.
35. **Rossmann, M. G., E. Arnold, E. A. Erickson, E. A. Frankenberger, J. P. Griffith, H.-J. Hecht, J. E. Johnson, and G. Kamer.** 1985. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature **317:**145–153.
36. **Rotbart, H. A.** 1989. Human enterovirus infections: molecular approaches to diagnosis and pathogenesis, p. 243–264. *In* B. L. Semler and E. Ehrenfeld (ed.), Molecular aspects of picornavirus infection and detection. American Society for Microbiology, Washington, D.C.
37. **Rozhon, E., S. Cox, P. Buontempo, J. O'Connell, W. Slater, J. DeMartino, J. Schwartz, G. Miller, E. Arnold, A. Zhang, C. Morrow, S. Jablonski, P. Pinto, R. Verace, T. Duelfer, and V. Girijavallabhan.** 1993. SCH 38057: a picornavirus capsid-binding molecule with antiviral activity after the initial stage of viral uncoating. Antivir. Res. **21:**15–35.
38. **Rozhon, E. J., H. L. Lipton, and F. Brown.** 1982. Characterization of Theiler's murine virus RNA. J. Gen. Virol. **61:**157–165.
39. **Rueckert, R. R., and M. A. Pallansch.** 1981. Preparation and characterization of encephalomyocarditis virus. Methods Enzymol. **78:**315–325.
40. **Rueckert, R. R.** 1990. Picornaviridae and their replication, p. 507–548. *In* B. N. Fields and D. M. Knipe (ed.), Virology. Raven Press, New York, N.Y.
41. **Schiff, G. M., J. R. Sherwood, E. C. Young, and L. J. Mason.** 1992. Prophylactic efficacy of WIN 54954 in prevention of experimental human coxsackievirus A21 infection and illness. Antivir. Res. **17**(Suppl. 1)**:**92.
42. **Staunton, D. E., V. J. Merluzzi, R. Rothlein, R. Barton, S. D. Marlin, and T. A. Springer.** 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell **56:**849–853.
43. **Strikas, R. A., L. J. Anderson, and R. A. Parker.** 1986. Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970–1983. J. Infect. Dis. **153:**346–351.
44. **Tomassini, J. E., D. Graham, C. M. DeWitt, D. W. Lineberger, J. A. Rodkey, and R. J. Colonno.** 1989. cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **86:**4907–4911.
45. **Trousdale, M. D., R. E. Paque, and C. J. Gauntt.** 1977. Isolation of coxsackievirus B3 temperature sensitive mutants and their assignment to complementation groups. Biochem. Biophys. Res. Commun. **76:**368–375.
46. **Turner, R. B., F. J. Dutko, N. H. Golstein, G. Lockwood, and F. G. Hayden.** 1993. Efficacy of oral WIN 54954 for prophylaxis of experimental rhinovirus infection. Antimicrob. Agents Chemother. **37:**297–300.
47. **Woods, M. G., G. D. Diana, M. C. Rogge, M. J. Otto, F. J. Dutko, and M. A. McKinlay.** 1989. In vitro and in vivo activities of WIN 54954, a new broad-

spectrum antipicornavirus drug. Antimicrob. Agents Chemother. **33:**2069–2074.
48. **Yin-Murphy, M.** 1984. Acute hemorrhagic conjunctivitis. Prog. Med. Virol. **29:**43–44.
49. **Zhang, A., R. G. Nanni, G. F. Arnold, D. A. Oren, T. Li, A. Jacobo-Molina, R. L. Williams, G. Kamer, D. A. Rubenstein, Y. Li, E. Rozhon, S. Cox, P. Buontempo, J. O'Connell, J. Schwartz, G. Miller, C. Nash, B. Bauer, R. Versace, A. Ganguly, V. Girijavallabhan, and E. Arnold.** 1991. Structure of a complex of human rhinovirus 14 with a water soluble antiviral compound SCH 38057. J. Mol. Biol. **230:**857–867.
50. **Zhang, A., R. G. Nanni, D. A. Oren, E. J. Rozhon, and E. Arnold.** 1992. Three-dimensional structure-activity relationships for antiviral agents that interact with picornavirus capsids. Semin. Virol. **3:**453–471.