

# Antiviral Activity in Tissue Culture Systems of bis-Benzimidazoles, Potent Inhibitors of Rhinoviruses<sup>1</sup>

J. B. SCHLEICHER, F. AQUINO, A. RUETER, W. R. RODERICK, AND R. N. APPELL  
*Abbott Laboratories, North Chicago, Illinois 60064*

Received for publication 30 July 1971

(*S,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol showed antiviral activity in monolayer tissue culture systems against 55 strains of rhinovirus, three types of poliovirus, and strains of type A and B coxsackieviruses. Neither the compound nor any of the analogues tested showed virucidal activity. Its antiviral activity was not associated with interference with viral attachment to or penetration into the cell. At a concentration of 0.1 mg/ml, this group of compounds was generally nontoxic to WI-38, primary bovine kidney, and African green monkey kidney cells and had antiviral activity with 100% inhibition of virus-induced cytopathic effects (CPE). At antiviral levels, these compounds prevented CPE of up to 10<sup>6</sup> median tissue culture infective dose units of virus and completely inhibited formation of new infective virions. The compounds showed antiviral activity both prophylactically and therapeutically against rhinoviruses. Infected cultures could be cleared of CPE up to 90 hr after infection.

(*S,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol (Abbott 36683), the structure of which is shown in Fig. 1, was reported to have antiviral activity against poliovirus type 1 in HeLa cells (1). Since poliovirus is a typical picornavirus, we investigated the antiviral activity of this compound against other members of the picornavirus group. Similar compounds, such as benzimidazole (2) and 2-( $\alpha$ -hydroxybenzyl)benzimidazole (HBB; references 3-5), 1,2-bis(2-benzimidazolyl)-1,2-ethanediol (7), and substituted benzimidazoles (6), have been reported to be active antiviral agents against an assortment of picornaviruses. Tamm and Eggers (8) recognized the considerable variation in the susceptibility of the picornavirus group to any single antiviral compound. This view was extended by Gwaltney's (4) suggestion that the D-HBB hydrochloride susceptibility of varying degree may be a common characteristic of all rhinoviruses. The results of testing Abbott 36683 contradicts many of the above theories, since this compound showed a wide spectrum of antiviral activity against the picornavirus group. However, analogues were more limited in their antiviral spectrum.

<sup>1</sup> Presented in part at the 10th Interscience Conference on Antimicrobial Agents and Chemotherapy, 18-21 October 1970, Chicago, Ill., Abstract 99.

## MATERIALS AND METHODS

**Tissue culture systems.** Diploid human embryonic lung (WI-38) cultures in tubes or flasks were grown with Eagle's basal medium (BME) plus 10% heat-inactivated fetal calf serum (FCS). Maintenance medium consisted of medium 199 supplemented with BME amino acids and vitamins plus 1 to 2% FCS. Plaque titrations of rhinovirus in WI-38 cells were performed with a methyl cellulose overlay medium. Primary bovine kidney cultures were grown in Earle's balanced salt solution (BSS) plus 0.5% lactalbumin hydrolysate plus 5% FCS (Melnick's growth medium) and were maintained in medium 199 plus 1% FCS. Primary African green monkey kidney cultures were grown in medium 199 plus 10% FCS and were maintained in medium 199 plus 1% FCS.

**Viruses.** Rhinovirus strains representative of the 55-numbered serotypes and one subtype were obtained from the Abbott Laboratories virus stocks. These had all previously been identified by specific antisera in neutralization tests. Poliovirus types I, II, and III were obtained from Naval Medical Research Unit 4. Coxsackievirus strains A9 and B3 were obtained from the National Institute of Allergy and Infectious Diseases. All virus stocks were further identified by serum neutralization tests versus known specific antisera.

**Experimental design.** Concentrations of each compound were tested against viral inocula of 32 to

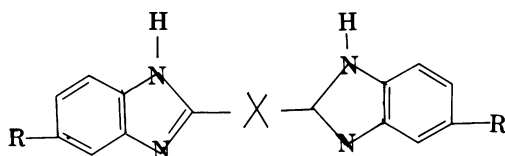


FIG. 1. Structure of (*S,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol.

100 median tissue culture infective dose units (TCID<sub>50</sub>). Testing was done in triplicate cell culture tubes. The viral inoculum was simultaneously titrated in medium without the test compound. Control tubes for each concentration of compound, without virus, were always included as toxicity controls. Tubes were incubated at 32 C for rhinoviruses and at 37 C for all other viruses, and were examined daily for cytopathic effects (CPE). Tests were judged completed when the control virus titration tubes showed 32 to 100 TCID<sub>50</sub> of challenge virus with clearly defined viral CPE. Comparisons were made at that time with the tubes containing virus-compound mixtures. The criterion of drug activity was complete suppression of CPE. The chemotherapeutic index (CTI), which is the highest nontoxic concentration of the compound divided by the lowest antiviral concentration, was determined for each compound and used as a measure of its antiviral activity. The usual concentrations tested were 1.0 to 0.01 mg per ml.

## RESULTS AND DISCUSSION

**Antiviral spectrum.** The results of testing human rhinovirus strains 1 to 55, poliovirus types I, II, and III, coxsackievirus types A9 and B3, and equine rhinovirus against Abbott 36683 are shown in Table 1. All 55 strains of human rhinoviruses were highly susceptible to this compound, with CTI values of 100 or greater. The compound was not toxic at 1.0 mg per ml and was active at 0.01 mg per ml. The compound was also active against the three poliovirus types and two types of coxsackievirus. The only picornavirus which showed considerable resistance was equine rhinovirus,

with a CTI value of 1. In other tests, not listed in Table 1, Abbott 36683 was not active against influenza, herpes-, vaccinia, respiratory syncytial (RS), parainfluenza, or vesicular stomatitis viruses in the tube test or in the plaque-inhibition test. Abbott 36683 showed no antiviral activity against equine rhinovirus in the plaque-inhibition test but showed excellent antiviral activity against human rhinoviruses by this method.

Several of the analogues of Abbott 36683 (Table 2) were tested by the tube method against several strains of human rhinovirus, coxsackieviruses A9 and B3, poliovirus type I, A<sub>2</sub>/170 influenza virus, RS virus, and herpesvirus. The results listed in Table 3 indicate a very broad spectrum of antipicornavirus activity for these compounds. Only the hydrogen chloride salt (Abbott 37536) of Abbott 36683 showed any activity outside the picornavirus group. It gave a CTI value of 3 against A<sub>2</sub>/170 influenza virus.

**Virucidal activity.** Decreasing concentrations of Abbott 36683, from 1.0 to 0.01 mg per

TABLE 1. Chemotherapeutic index<sup>a</sup> of Abbott 36683 against several picornaviruses

Virus	Cells		
	WI-38	Monkey kidney	Bovine kidney
Rhinovirus			
Strains 1-55 . . .	≥ 100		
Equine . . . . .		1	
Poliovirus			
Type I . . . . .	100	100	
Type II . . . . .	32	100	
Type III . . . . .	100	100	
Coxsackievirus			
A9 . . . . .		100	
B3 . . . . .			10

<sup>a</sup> Highest nontoxic concentration per lowest antiviral concentration.

TABLE 2. Analogues of Abbott 36683

Compound <sup>a</sup>	Configuration	R	X	
A-36683	( <i>S,S</i> )	OCH <sub>3</sub>	(CH—OH) <sub>2</sub>	
A-37984	( <i>S,S</i> )	OC <sub>2</sub> H <sub>5</sub>	(CH—OH) <sub>2</sub>	
A-37985	( <i>S,S</i> )	OC <sub>2</sub> H <sub>5</sub>	(CH—OH) <sub>2</sub>	dihydrochloride salt
A-37536	( <i>S,S</i> )	OCH <sub>3</sub>	(CH—OH) <sub>2</sub>	dihydrochloride salt
A-37593	( <i>R,S</i> )	OCH <sub>3</sub>	(CH—OH) <sub>2</sub>	
A-37594	( <i>R,R</i> )	OCH <sub>3</sub>	(CH—OH) <sub>2</sub>	
A-37595		OCH <sub>3</sub>	(CH <sub>2</sub> ) <sub>2</sub>	

<sup>a</sup> Compounds represent, respectively: A-36683, (*S,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol; A-37984, (*S,S*)-1,2-bis(5-ethoxy-2-benzimidazolyl)-1,2-ethanediol; A-37985, (*S,S*)-1,2-bis(5-ethoxy-2-benzimidazolyl)-1,2-ethanediol dihydrochloride; A-37536, (*S,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol dihydrochloride; A-37593, (*R,S*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol; A-37594, (*R,R*)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol; A-37595, 1,2-bis(5-methoxy-2-benzimidazolyl)ethane.

TABLE 3. Antiviral chemotherapeutic index<sup>a</sup> of analogues of Abbott 36683

Virus	Compound <sup>b</sup>					
	A-37984	A-37985 (HCl)	A-37536 (HCl)	A-37593 (HCl)	A-37594 (HCl)	A-37595 (HCl)
Rhinovirus						
Strain 1A	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
Strain 13	≥ 100	≥ 100				
Strain 14	≥ 100	≥ 100				
Strain 16	≥ 100	≥ 100				
Strain 17	100	≥ 100				
Strain 24			≥ 100	≥ 100		
Strain 25			≥ 100	≥ 100		
Strain 26			≥ 100	10		
Strain 27	≥ 100	≥ 100				
Strain 28	≥ 100	≥ 100				
Strain 29			≥ 100			
Strain 32	≥ 100	32				
Strain 34	≥ 100	≥ 100				
Strain 42	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
Strain 44	≥ 100					
Strain 50	≥ 100	≥ 32				
Strain 51	≥ 100	≥ 32				
Strain 52	≥ 100	≥ 100				
Strain 53	≥ 100	≥ 100				
Strain 54	≥ 100	10				
Strain 55	≥ 100	10				
Coxsackievirus A9	≥ 100	≥ 100	≥ 100	3	100	100
Coxsackievirus B3	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
Poliovirus type I	≥ 100	≥ 100	≥ 100	32	≥ 100	10
Influenza A2/170	0	0	3	0	0	0
Respiratory syncytial	0	0	0	0	0	0
Herpesvirus	0	0	0	0	0	0

<sup>a</sup> Highest nontoxic concentration per lowest antiviral concentration.

<sup>b</sup> Numbers prefixed by A represent the Abbott laboratories designations of the compounds. The highest drug concentration tested was 1.0 mg/ml.

TABLE 4. Chemotherapeutic activity of Abbott 36683 against rhinovirus strain 1A

Time after addition of virus (hr)	Lowest active concn of drug (μg/ml)	CPE of control tubes at time of compound addition <sup>a</sup>
0	0.1	0
8	1.0	0
24	10	±
32	10	1+
48	10	2+
72	10	3+
96	10	3 to 4+

<sup>a</sup> Cytopathic effect: 0, no CPE; ±, one focus of CPE; 1+, ca. 25% of cells involved with CPE; 2+, ca. 50% of cells involved with CPE; 3+, ca. 75% of cells involved with CPE; 4+, ca. 100% of cells involved with CPE.

ml, were mixed with an equal volume of undiluted rhinovirus strain 1A and incubated at room temperature, one set for 2 hr and one set for 8 hr. Each mixture, as well as a control

virus in Earle's BSS without compound, was titrated in WI-38 tubes and incubated at 32 C for 7 days. Abbott 36683 had no virucidal activity even after 8 hr of contact with the virus. In a series of control tubes inoculated with 10<sup>6.5</sup> TCID<sub>50</sub> of virus and various drug concentrations, but not titrated, no CPE developed.

**Chemotherapeutic activity.** Rhinovirus strain 1A was inoculated into a series of WI-38 tubes, and, at various times after addition of virus, various concentrations of Abbott 36683 were added to sets of these tubes. All tubes were incubated at 32 C and inspected for CPE daily. The results listed in Table 4 indicate that Abbott 36683 can be added as late as 96 hr after the virus and still show antiviral activity with 10 μg per ml, even after viral CPE had advanced to a 3+ stage (75% of sheet exhibiting CPE).

**Mechanism of action.** An experiment was designed to determine whether the compound interfered with the attachment or penetration of the virus into the cell. Rhinovirus strain 1A

was used at 100 TCID<sub>50</sub> per tube. Specific antiserum which had a reciprocal titer of 1:1,000 was used at a 1:50 dilution. The compound, Abbott 36683, at concentrations of 10, 1.0, and 0.1  $\mu$ g per ml, was added to two sets of WI-38 tissue culture tubes. Virus was added, and the tubes were incubated for 2 hr at room temperature. Antiserum to rhinovirus strain 1A was then added to one set of tubes. The other set received only medium as a control. All tubes were again incubated for 90 min at room temperature. The cells in half of the tubes in each group were washed twice with maintenance medium, and fresh medium was replaced prior to reincubation at 32 C for several days. Daily observations were made. The results showed that in each group of tubes in which the compound was washed out of the system, virus reproduced and caused a typical CPE. Where Abbott 36683 remained, no CPE was observed. The presence of antiserum had no effect on the presence or absence of viral CPE.

Since 20 units of specific antiserum which had a reciprocal neutralization titer of 1:1,000 had no effect on the virus after preincubation for 2 hr with the compound, the virus must have had time to attach to and penetrate the cell. Thus, the mechanism of action of Abbott 36683 must be at a site other than the surface of the cell where attachment and penetration occur, and at a stage of the viral replicative cycle subsequent to these early events.

The broad spectrum of antiviral activity of Abbott 36683 bis-benzimidazole versus rhinoviruses was demonstrated along with that of several related analogues. The very high antiviral activity and absences of toxicity in tissue culture suggested that this drug might be a candidate for possible use in man's battle with the "common cold."

#### LITERATURE CITED

1. Akihama, S., M. Okude, K. Sata, and S. Iwabuchi. 1968. Inhibitory effect of 1,2-bis(2-benzimidazolyl)-1,2-ethanediol derivatives on poliovirus. *Nature (London)* **217**:562-563.
2. Brown, G. C. 1952. The influence of chemicals on the propagation of poliomyelitis virus in tissue culture. *J. Immunol.* **69**:441-450.
3. Eggers, H. J., and I. Tamm. 1966. *Annu. Rev. Pharmacol.* **6**:231-250.
4. Gwaltney, J. M., Jr. 1968. The spectrum of rhinovirus inhibition by 2-(alpha-hydroxybenzyl)-benzimidazole and D-(-)-2-(alpha-hydroxybenzyl)-benzimidazole HCl. *Proc. Soc. Exp. Biol. Med.* **129**:665-673.
5. Hollinshead, A. C., and P. K. Smith. 1958. Effect of certain purines and related compounds on virus propagation. *J. Pharmacol. Exp. Ther.* **123**:54-62.
6. O'Sullivan, D. G., C. Ludlow, D. Pantic, and A. K. Wallis. 1970. Antiviral activity and molecular features of benzimidazoles. *Antimicrob. Ag. Chemother.* **1969**, p. 153-159.
7. O'Sullivan, D. G., and A. K. Wallis. 1963. New benzimidazole derivatives with powerful protective action on tissue-culture cells infected with types 1, 2, and 3 poliovirus. *Nature (London)* **198**:1270-1273.
8. Tamm, I., and H. J. Eggers. 1962. Differences in the selective inhibitory action of 2-(alpha-hydroxybenzyl)-benzimidazole and guanidine HCl. *Virology* **18**:439.