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Antipicornavirus activity of some diaryl methanes and aralkylaminopyridines

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Summary

Sixteen diarylmethanes and ten aralkylaminopyridines were initially evaluated for their in vitro activity against rhinoviruses 1A, 2 and 64 and against coxsackievirus A21 and for their oral prophylactic and therapeutic activity in mice challenged with coxsackievirus A21. Based on these preliminary studies the diarylmethane (3,4-dichlorophenoxy)-(5 methylsulfonyl-2-pyridinyl)-methane and the aralkylaminopyridine 2-(3,4-dichlorobenzylamino)-5-methylsulfonylpyridine were compared with their oxygen bridged analogue 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine for in vitro activity against a larger number of picornaviruses and for their in vivo protective efficacy in dose response assays. All three compounds exhibit similar in vitro activity inhibiting 12 to 15 (52.2–65.3%) of the 23 picornaviruses tested at concentrations of < 5.0 μg/ml. However, the aralkylaminopyridine was found to be the most active in vivo; significantly protecting coxsackievirus A21 challenged mice after a single oral dose of 37.5 mg/kg ($P \leq 0.05$) and during a continuous oral dose regimen of as low as 18.8 mg/kg per day ($P < 0.01$).

diaryl methanes; aralkylaminopyridines; picornaviruses; enteroviruses; rhinoviruses

Introduction

In earlier studies (Kenny et al., 1985) we showed that phenoxypyridines substituted with a sulfur-containing alkyl moiety and a cyano group on the pyridine ring

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coupled with a 3,4-dichloro group on the benzene ring possess good in vitro rhinovirus activity. Replacement of the 3,4-dichloro group with a *para*-benzoyl moiety reduced rhinovirus activity but tended to improve overall in vitro and in vivo enterovirus activity (Kenny et al., 1986). Subsequently we explored variations in the aryl bridging group and the results of those studies are summarized in this communication.

Materials and Methods

Test compounds

The diarylmethanes (Table 1) and the diaryl ether, 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine (Fig. 1), were synthesized by Y. Tong and S.G. Wood, The Dow Chemical Co., Walnut Creek, CA, while the aralkylaminopyridines (Table 2) were prepared by two of us (T.M. Bargar and J.K. Daniel).

Test viruses

The following viruses were purchased from the American Type Culture Collection (ATCC), Rockville, MD: rhinovirus types 1B (ATCC VR-1111), 4 (ATCC VR-484), 9 (ATCC VR-489), 10(ATCC VR-490), 13 (ATCC VR-286), 21 (ATCC VR-496), 29 (ATCC VR-504), 33(ATCC VR-1143), 44(ATCC VR-1154), 68 (ATCC VR-1178), 74 (ATCC VR-1184) and 89 (ATCC VR-1199). Rhinovirus types 5, 8, 39, 64 and Hanks were supplied by J. Gwaltney, University of Virginia School of Medicine, Charlottesville, VA, while rhinovirus types 1A and 2 were obtained from B.D. Korant, E.I. DuPont de Nemours and Company, Wilmington, DE. A11 rhinovirus experimental stocks were prepared in HeLa cells. Coxsackievirus A21, supplied by D.C. DeLong, Eli Lilly and Co., Indianapolis, IN, was grown in weanling mice and then passed once in HeLa cells. Coxsackievirus B3, obtained from C. Gauntt, University of Texas Health Science Center, Dallas, TX; coxsackievirus B4, supplied by G. Burch, Tulane University Medical School, New Orleans, LA; and echovirus 12, obtained from G. Schiff, The James N. Gamble Institute of Medical Research, Cincinnati, OH, were grown in Vero cells and then adapted to HeLa cells.

Cell culture

HeLa and Vero cells grown and maintained at 36°C in Corning 75-cm^2 tissue culture flasks (Scientific Products, McGaw Park, IL) using Eagle's minimal essential medium (EMEM, GIBCO Grand Island, NY) supplemented with 50 μg penicillin G, 50 μg streptomycin sulfate and 100 μg neomycin sulfate per ml (1% PSN,

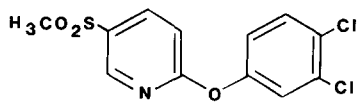


Fig. 1. Structure of 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine.

GIBCO). 7–10% heat inactivated fetal bovine serum (HIFBS, MA Bioproducts, Walkersville, MD) was added to the medium for cell growth (growth medium) and the concentration was reduced to 1–2% for cell maintenance (maintenance medium). For in vitro assays HeLa cells were transferred to 24 well microtiter plates (Costar, Cambridge, MA) at a concentration of $1.0–1.3 \times 10^5$ cells per well in 1.0 ml growth medium. After 24 h growth at 36°C in a humidified CO_2 (5% CO_2/95% air) incubator the cultures were 60–75% monolayered and ready for use.

In vitro assessment of antiviral activity

For each assay 2 mg of test compound was mixed with 0.02 ml of dimethyl sulfoxide (DMSO, Matheson, Coleman and Bell) followed by the addition of 10 ml maintenance medium to give a concentration of 200 μg compound per ml. Dilutions were then prepared in the same medium to give test concentrations of 0.3–10 μg compound per ml. Cell cultures in the 24 well microtiter plates were drained and refed with 1.0 ml compound-containing or compound-free maintenance medium. Appropriate monolayers (except compound toxicity and cell controls) were then challenged with 0.1 ml (10–100 $TCID_{50}$) virus. The cultures were then incubated at 33 or 36°C in a humidified CO_2 incubator and examined microscopically at 48, 72 and 96 h for compound cytotoxicity and virus cytopathic effect (CPE). The lowest concentration of compound that reduced viral CPE by 50% or more was considered to be the minimum inhibitory concentration (MIC_{50}). A standard control compound, 6-(4-nitrophenoxy)-3-pyridinecarbonitrile, was also run in each assay. Control compound MIC_{50} values for rhinoviruses 1A and 2, and for coxsackievirus A21 ranged from 3.1 to 6.3 μg/ml for all assays.

Cell growth inhibition

The effect of test compounds on the development of isolated HeLa cell colonies was determined by the techniques described previously (Torney et al., 1982).

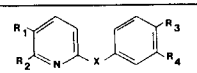
Compound preparation for in vivo assays

For the single oral dose test, compounds were suspended in 0.5% aqueous hydroxypropylmethyl cellulose (Methocel® type MC Premium, viscosity 15 cps, Dow Chemical USA, Midland, MI) at a concentration of 0.94–30 mg/ml. Uniform suspension was accomplished by homogenization. Oral gavage with 0.2 ml of homogenate resulted in a compound dose of 18.8–600 mg/kg mouse weight. Control animals received 0.2 ml compound-free 0.5% aqueous Methocel by the same route.

For the preparation of 0.0075–0.06% compound-containing food, 0.45–3.6 g of each compound was dissolved in 27 ml acetone and then admixed with 79.2 g silica gel (Hi-Sil 233, Pittsburg Plate Glass Inds., Pittsburg, PA). After acetone evaporation, the gel/compound complex was mixed with 6.0 kg ground mouse food (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) on an automatic roller (Dayton/Paul Abbe, Inc., Brooklyn, NY) for 1–3 h. The resultant compound-food mixture, when fed ad libitum to 9–12 g (19–20-day-old) mice, results in a daily compound dose of approximately 9.4–75 mg/kg mouse weight (Lehman et al., 1950).

TABLE 1

In vitro picornavirus activity of some diaryl methanes (X = CH_2)

Compound	R_1	R_2	R_3	R_4	Lowest concentration (in μg/ml) that caused:				
					Cytotoxicity	≥50% reduction in CPE			
						RV-1A	RV-2	RV-64	COX A21
1	SO_2CH_3	H	H	H	>10	>10	>10	>10	>10
2	SO_2CH_3	H	Br	H	>10	0.6	2.5	0.6	5.0
3	SO_2CH_3	H	F	H	>10	2.5	>10	>10	2.5
4	SO_2CH_3	H	CH_3	H	>10	5.0	>10	5.0	>10
5	SO_2CH_3	H	NH_2	H	>10	>10	>10	>10	>10
6	SO_2CH_3	H	$N(CH_3)_2$	H	>10	>10	>10	>10	>10
7	SO_2CH_3	H	$NHCOCH_3$	H	>10	>10	>10	>10	>10
8	SO_2CH_3	H	SCH_3	H	>10	5	5	1.3	>10
9	SO_2CH_3	H	$SOCH_3$	H	>10	>10	>10	>10	>10
10	SO_2CH_3	H	SO_2CH_3	H	>10	2.5	>10	>10	>10
11	SO_2CH_3	H	*S*-Phenyl	H	2.5	0.6	1.3	1.3	<0.6
12	SO_2CH_3	H	Cl	H	>10	0.3	2.5	1.3	2.5
13	SO_2CH_3	H	Cl	Cl	>10	<0.3	0.6	<0.3	5.0
14	$SO_2CH_2CH_3$	H	Cl	Cl	>10	0.6	1.3	0.6	1.3
15	$SO_2CH(CH_3)_2$	H	Cl	Cl	10	<0.3	1.3	0.6	0.6
16	$SO_2CH_2CH_3$	$CONH_2$	Cl	Cl	10	1.3	2.5	2.5	5.0

In vivo assessment of antiviral activity

In vivo antiviral activity was evaluated in 19–20-day-old random bred Swiss albino mice (Harlan Sprague Dawley, Inc., Indianapolis, IN). In the single dose antiviral tests mice were challenged i.p. with 0.2 ml coxsackievirus A21 3 h before administration of the compound as an oral gavage. For the continuous dose test, animals were placed on compound-containing or placebo food 24 h before i.p. virus challenge. Compound-containing or placebo food was then continued ad libitum throughout the test period. In these assays the coxsackievirus A21 was diluted to a concentration sufficient to cause 75–100% mortality of infected control mice within 9 days of an i.p. injection of 0.2 ml virus. All mice were observed for 8–9 days for deaths. A modified Mantel-Haenszel combined chi-square analysis (Mantel, 1963) was then used to determine the differences between treated and placebo groups. This statistical analysis incorporates both the day of death and the number of survivors into a single variable to measure compound efficacy. All compounds were initially screened in both assays at one concentration. Selected active compounds were then re-evaluated in a dose response assay. In these studies, groups of 15 to 33 mice were used for each test dose.

Results

Preliminary screen for in vitro and in vivo activity and for cytotoxicity

The preliminary in vitro evaluations of a series of 5-alkylsulfonyl substituted diarylmethanes and aralkylaminopyridines are summarized in Tables 1 and 2, respectively. These studies show that a 3′, 4′-dichloro substitution on the benzene moiety (compounds 13, 14, 15, 21, 26) is associated with the best overall antipicornavirus activity. No benzene substitution (compound 1) or para substitution of the benzene moiety with methyl (compound 4), amino (compound 5), dimethylamino (compound 6), aminoacetyl (compound 7), methylsulfoxide (compound 9), methylsulfone (compound 10), chloro (compounds 12 and 18), or a 3′,4′-methoxy (compound 20) group greatly reduces or eliminates in vitro antipicornavirus activity. Initial in vivo studies (Tables 3, 4) showed that a para or a 3′,4′-halogen group on the benzene moiety (compounds 2, 3, 12, 13, 14, 17, 18, 19, 21, 22, 23, 24, 26) also is associated with significant protection against a lethal i.p. challenge of coxsackievirus A21.

The aralkylaminopyridines studied here were devoid of detectable HeLa cell cytotoxicity whereas three of the diarylmethanes (compounds 11, 15, 16) produced cytotoxicity at concentrations of 2.5–10.0 μg/ml.

Expanded spectrum of in vitro activity

Based on these initial findings the diaryl methane (3,4-dichlorophenoxy)-(5-methylsulfonyl-2-pyridinyl)-methane (compound 13) and the aralkylaminopyridine 2-(3,4-dichlorobenzylamino)-5-methylsulfonylpyridine (compound 21) were compared with their oxygen bridged analogue 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine (Markley et al., 1986) (Fig. 1) against a larger number of pi-

TABLE 2

In vitro picornavirus activity of some aralkylaminopyridines

Compound	R_1	R_2	X	R_3	R_4	Lowest concentration (in μg/ml) that caused:				
						Cytotoxicity	>50% reduction in CPE			
							RV-1A	RV-2	RV-64	COX A21
17	SO_2CH_3	H	$NHCH_2$	Br	H	>10	>10	6.3	6.3	1.6
18	SO_2CH_3	H	$NHCH_2$	Cl	H	>10	3.1	3.1	1.6	1.6
19	SO_2CH_3	H	$NHCH_2$	CF_3	H	>10	>10	6.3	1.6	< 1.6
20	SO_2CH_3	H	$NHCH_2$	OCH_3	OCH_3	>10	>10	>10	>10	>10
21	SO_2CH_3	H	$NHCH_2$	Cl	Cl	>10	3.1	0.6	0.3	0.6
22	$SO_2CH_2CH_3$	H	$NHCH_2$	Cl	Cl	>10	>10	>10	6.3	>10
23	$SO_2CH_2CH_3$	CN	$NHCH_2$	Cl	Cl	>10	3.1	0.6	0.6	2.5
24	$SO_2CH_2CH_3$	CN	$NHCH_2$	Cl	H	>10	6.3	1.6	6.3	1.6
25	SO_2CH_3	H	$NHCH_2CH_2$	Cl	H	>10	3.1	3.1	1.6	>10
26	SO_2CH_3	H	$NHCH_2CH_2$	Cl	Cl	>10	< 1.3	< 1.3	< 1.3	5.0

TABLE 3

In vivo activity of some diaryl methanes in the single oral dose and feed tests

Test	Compound	Dose[a]	Test day						Combined chi square[c]	Level of significance (P)
			4	5	6	7	8	9		
Single oral dose	2	300 mg/kg	30/30:30/30[b]	28/30:28/30	25/30:20/30	22/30:12/30	22/30:8/30	19/30:4/30	14.79	<0.001
	3	300 mg/kg	30/30:30/30	30/30:28/30	28/30:20/30	24/30:12/30	23/30:8/30	21/30:4/30	21.12	<0.001
	12	600 mg/kg	29/30:30/30	20/30:19/30	25/30:10/30	20/30:3/30	8/30:2/30	18/30:2/30	26.53	<0.001
	13	600 mg/kg	30/30:30/30	29/30:19/30	23/30:10/30	17/30:3/30	11/30:2/30	11/30:2/30	16.83	<0.001
	14	600 mg/kg	30/30:30/30	27/30:28/30	25/30:20/30	19/30:12/30	18/30:8/30	15/30:4/30	8.36	<0.01
Feed	12	75 mg/kg/day	28/30:31/31	25/30:18/31	12/30:6/31	10/30:4/31	10/30:4/31	10/30:4/31	5.58	<0.02
	13	75 mg/kg/day	31/32:31/32	30/32:15/32	26/32:3/32	19/32:1/32	19/32:1/32	19/32:1/32	39.43	<0.001

[a] Highest dose tolerated as determined in preliminary toxicity studies.
[b] Survivors/total for test compound: survivors over total for placebo.
[c] Based on modified Mantel-Haenszel procedure with 1 degree of freedom.

TABLE 4

In vivo activity of some aralkylaminopyridines in the single oral dose and feed tests

Test	Compound	Dose[a]	Test day						Combined chi square[c]	Level of significance (P)
			4	5	6	7	8	9		
Single oral	17	600 mg/kg	29/30:28/30[b]	25/30:13/30	17/30:6/30	11/30:4/30	10/30:3/30	10/30:2/30	10.61	<0.01
	18	600 mg/kg	30/30:29/30	19/30:14/30	12/30:3/30	6/30:1/30	2/30:0/30		6.69	<0.01
dose	19	600 mg/kg	29/30:28/30	28/30:13/30	26/30:6/30	26/30:4/30	26/30:3/30	25/30:2/30	39.91	<0.001
	21	600 mg/kg	29/30:29/30	29/30:14/30	29/30:3/30	27/30:1/30	18/30:0/30		51.85	<0.001
	22	600 mg/kg	30/30:30/30	30/30:26/30	27/30:21/30	18/30:7/30	15/30:4/30	10/30:2/30	11.07	<0.001
	23	600 mg/kg	30/30:30/30	26/30:23/30	11/30:5/30	2/30:0/30			3.96	<0.05
	24	400 mg/kg	29/30:30/30	25/30:23/30	14/30:5/30	6/30:0/30			7.19	<0.01
	26	600 mg/kg	29/30:28/30	27/30:20/30	17/30:14/30	10/30:4/30	10/30:3/30	10/30:3/30	4.44	<0.05
Feed	19	75 mg/kg/day	15/15:15/15	15/15:11/15	15/15:7/15	15/15:3/15	15/15:2/15	15/15:0/15	33.29	<0.001
test	21	75 mg/kg/day	33/33:30/33	33/33:19/33	32/33:10/33	31/33:6/33	29/33:4/33	29/33:4/33	43.33	<0.001

[a] Highest dose tolerated as determined in preliminary toxicity studies.

[b] Survivors/total for test compound: survivors/total for placebo.

[c] Based on modified Mantel-Haenszel procedure with 1 degree of freedom.

TABLE 5

Expanded spectrum of in vitro anti-picornavirus activity of compounds 13 and 21 compared to their oxygen bridged analogue 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine

Virus		MIC_{50} (in μg/ml)		
		Cpd 13	Cpd 21	Analogue
Rhinovirus	1A	0.3	2.5	0.3
	1B	<0.3	0.6	<0.3
	2	0.6	0.6	0.6
	4	>10.0	>10.0	>10.0
	5	>10.0	>10.0	>10.0
	8	>10.0	>10.0	>10.0
	9	>10.0	5.0	10.0
	10	2.5	2.5	1.3
	13	>10.0	>10.0	>10.0
	21	>10.0	5.0	10.0
	29	2.5	1.3	1.3
	33	1.3	0.6	0.6
	39	5.0	5.0	3.1
	44	5.0	2.5	5.0
	64	0.3	0.3	0.5
	68	>10.0	>10.0	>10.0
	74	5.0	1.3	2.5
	89	1.3	1.3	1.3
	Hank's	>10.0	5.0	6.3
Echovirus	12	6.3	>10.0	1.3
Coxsackievirus	A21	5.0	0.6	6.3
	B3	>10.0	>10.0	>10.0
	B4	>10.0	>10.0	6.3

cornaviruses. Table 5 shows that the 3 compounds exhibit a similar spectrum of in vitro activity inhibiting 12–15 (52.2–65.3%) of the 23 picornaviruses tested at concentrations of $\leq$ 5.0 μg/ml.

Dose-response assays

The ability of compounds 13 (methylene bridged) and 21 (aminoalkyl bridged) and their oxygen bridged analogue to protect mice lethally infected with coxsackievirus A21 was compared in dose response assays. The results are summarized in Table 6. All three compounds are highly active ($P < 0.001$) when given as a single oral dose of 150 mg/kg 3 h after i.p. virus challenge. In addition, compounds 21 and 13 show significant ($P < 0.05$) activity when given as a single oral dose of 37.5 mg/kg and 75.0 mg/kg, respectively. Compound 21 also shows the greatest protective efficacy when administered continuously in the food. Significant ($P < 0.01$) antiviral activity is observed at a dose of as low as 18.8 mg/kg per day. In contrast, a dose of 75.0 mg of compound 13 per kg per day ($P < 0.02$) or a dose of 37.5 mg oxygen bridged analogue per kg per day ($P < 0.05$) is required to significantly enhance survival of coxsackievirus A21-infected mice when compared to the placebo-treated controls.

TABLE 6

In vivo response of compounds 13 and 21 and their oxygen-bridged analogue administered as a single oral dose or in the food

Test	Compound	Dose	Test day						Combined chi square[b]	Level of significance (P)
			4	5	6	7	8	9		
Single oral dose	13	150 mg/kg	30/30[a]	29/30	15/30	2/30	1/30	1/30	13.94	<0.001
		75 mg/kg	26/30	21/30	13/30	3/30	2/30	2/30	4.6	<0.05
		37.5 mg/kg	26/30	14/30	7/30	3/30	2/30	2/30	0.79	Not active
		Placebo	24/30	9/30	3/30	3/30	2/30	2/30		
	21	150 mg/kg	30/30	22/30	16/30	12/30	10/30	7/30	14.41	<0.001
		75 mg/kg	30/30	28/30	16/30	4/30	2/30	2/30	13.08	<0.001
		37.5 mg/kg	30/30	21/30	8/30	2/30	2/30	2/30	5.20	<0.05
		18.8 mg/kg	28/30	16/30	6/30	3/30	1/30	1/30	1.17	Not active
		Placebo	24/30	9/30	3/30	3/30	2/30	2/30		
	Analogue	300 mg/kg	25/25	23/25	21/25	19/25	18/25	18/25	17.35	<0.001
		150 mg/kg	25/25	25/25	23/25	21/25	18/25	17/25	21.06	<0.001
		75 mg/kg	25/25	22/25	18/25	10/25	4/25	2/25	0.60	Not active
		Placebo	23/25	23/25	12/25	5/25	4/25	3/25		
Feed	13	75 mg/kg[c]	28/30	25/30	12/30	10/30	10/30	10/30	5.58	<0.02
		37.5 mg/kg[c]	30/31	21/31	10/31	5/31	5/31	5/31	0.72	Not active
		Placebo	31/31	18/31	6/31	4/31	4/31	4/31		
	21	37.5 mg/kg[c]	33/33	32/33	25/33	20/33	18/33	18/33	20.31	<0.001
		18.8 mg/kg[c]	32/33	27/33	20/33	17/33	11/33	11/33	7.66	<0.01
		9.4 mg/kg[c]	28/33	21/33	8/33	3/33	3/33	3/33	0.13	Not active
		Placebo	30/33	19/33	10/33	6/33	4/33	4/33		
	Analogue	75 mg/kg[c]	28/29	24/29	23/29	19/29	17/29	17/29	6.77	<0.01
		37.5 mg/kg[c]	30/30	27/30	22/30	16/30	15/30	15/30	4.76	<0.05
		18.8 mg/kg[c]	28/30	23/30	18/30	15/30	11/30	11/30	0.79	Not active
		Placebo	30/30	26/30	13/30	10/30	7/30	7/30		

[a] Number of survivors/total number.

[b] Based on modified Mantel-Haenszel procedure with one degree of freedom.

[c] Dose per day.

Host cell effects

Compound 21 inhibits development of isolated HeLa cell colonies at concentrations of as low as 12.5 μg/ml whereas HeLa cell growth is inhibited by compound 13 and the oxygen bridged analogue at concentrations of 12.5–25 μg/ml.

Discussion

The picornaviruses are responsible for a wide spectrum of human diseases including encephalitis, aseptic meningitis, myocarditis, pericarditis, hepatitis, and upper respiratory tract disease. Except for the polioviruses, and perhaps hepatitis A, development of antipicornavirus vaccines is not practical because of the existence of a large number of serotypes. As a result, we have attempted to develop compounds possessing broad spectrum antipicornavirus activity after oral administration. The synthesis and evaluation of a large number of phenoxybenzenes and phenoxypyridines has shown that many diarylethers possessing one electron-deficient ring demonstrate good antipicornavirus activity (Markley et al., 1986). Within the phenoxybenzene series, compounds possessing a 2-CN-4-NO_2 or a 3,4-dichloro substituent show the greatest activity. One of these compounds, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile, has been studied extensively and shown to be a potent antipicornavirus compound (Powers et al., 1982; Torney et al., 1982). In an attempt to maintain optimal structural criteria and to avoid potential toxicity associated with aromatic nitro-compounds we synthesized a series of 3,4-dichlorophenoxypyridinecarbonitriles. Evaluation of these compounds (Kenny et al., 1985) showed that replacement of one of the benzene rings with analogues containing an electron-deficient pyridine ring substituted with an electron-withdrawing cyano group and a sulfur-containing alkyl moiety did not improve antipicornavirus activity. Elimination of the cyano group results in improved rhinovirus activity but enterovirus activity is further diminished (Kenny et al., 1986; Markley et al., 1986). Replacement of the 3,4-dichloro group on the benzene moiety by a *para*-benzoyl group did not improve rhinovirus activity but several analogues in the series demonstrated increased in vitro and in vivo enterovirus activity (Kenny et al., 1986). In the latest series of analogues we have examined replacement of the oxygen with a methylene or aminoalkyl bridging group. The most active compounds are those possessing a 3,4-dichloro substitution on the benzene ring in combination with a 5-alkyl sulfur group on the pyridine ring thus confirming earlier observations on the necessity of this combination of aryl substituents (Kenny et al., 1985; Markley et al., 1986). Replacement of oxygen with a methane or alkylamino bridging group has little effect on in vitro antipicornavirus activity, but an alkylamino bridging group slightly enhances protective efficacy in mice lethally infected with coxsackievirus A21. Finally, introduction of an aminoalkyl bridging group increases cell toxicity as evidenced by the inhibition of the development of isolated HeLa cell colonies.

In summary, the in vitro and in vivo activity of the title compounds coupled with their effect on cell growth limits their potential clinical application to the most serous picornavirus infections.

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