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In vitro and in vivo anti-picornavirus activity of some *p*-benzoylphenoxypyridines

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Summary

Fifteen *p*-benzoylphenoxypyridines were initially evaluated for their in vitro activity against rhinoviruses (RV) 1A, 2 and 64 and coxsackie virus (Cox) A21 and for their oral prophylactic and therapeutic activity in Swiss albino mice lethally challenged with Cox A21. One compound, {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone, was selected for additional evaluation. These studies showed the compound to possess MIC_{50} values of $\leqslant 5$ μg/ml against only 6 of 20 (30.0%) RV serotypes tested. In contrast, the compound was active at concentrations of $\leqslant 5.0$ μg/ml against 10 of 12 (83.3%) enteroviruses evaluated. In vivo studies showed the compound to significantly protect mice lethally infected with Cox A21 after a single oral dose of 37.5 mg/kg ($P < 0.02$) and during a regimen of continuous oral doses of at least 4.7 mg/kg per day ($P < 0.001$). Mechanism of action studies indicated that the compound inhibits picornavirus uncoating or some earlier virus-host cell-associated event. Isotopic studies show that {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone perturbs HeLa cell macromolecular synthesis at concentrations of as low as 3.12 μg/ml. This concentration is only 4-fold higher than the concentration of compound necessary to inhibit Cox A21 RNA synthesis by 90%. This narrow therapeutic ratio limits the potential clinical utility of this compound to all but the most serious picornavirus infections.

p-benzoylphenoxypyridines; picornaviruses; enteroviruses; rhinoviruses

Introduction

Attempts to develop orally active broad spectrum anti-picornavirus compounds

have led our laboratories to synthesize and evaluate a large number of phenoxybenzenes and phenoxypyridines. Earlier studies [14] showed that anti-picornavirus activity requires electron deficient aromatic rings and that compounds possessing electron withdrawing groups such as 2-cyano-4-nitro and 3,4-dichloro substitutions possess the best overall activity. One of these compounds, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile, was extensively evaluated and shown to be a potent anti-picornavirus compound [21,30]. Further attempts to maintain optimal structural criteria and to avoid potential toxicity associated with such aromatic nitrocompounds led us to the synthesis of a series of 3,4-dichlorophenoxypyridinecarbonitriles. The evaluation of these compounds is discussed in a separate communication [8]. Other studies [14; this communication] showed that a 5-methylsulfonyl substituted 2-(3,4-dichlorophenoxy)pyridine (Fig. 1) possesses good anti-rhinovirus (RV) activity but is relatively devoid of enterovirus activity. In attempts to increase the enterovirus activity and to maintain or improve RV activity a series of *p*-benzoylphenoxypyridines were synthesized and evaluated for their overall anti-picornavirus activity. These studies are summarized here.

Materials and Methods

Test compounds

The compounds evaluated here were synthesized by Y.C. Tong and S.G. Wood, Western Division Research Center, The Dow Chemical Company.

Test viruses

Coxsackievirus (Cox) A9 (ATCC VR-1015), echoviruses (ECHO) 6 (ATCC VR-1044), 14 (ATCC VR-1055), 19 (ATCC VR-1060) and 30 (ATCC VR-1072) were obtained from The American Type Culture Collection (ATCC), Rockville, MD. Virus stocks were prepared in African Green monkey kidney cells. The origin and passage history of the other viruses used in these studies have been detailed in an earlier publication [8].

Tissue culture

HeLa (GIBCO Laboratories, Grand Island, NY), Vero (MA Bioproducts, Walkersville, MD), MRC-5 (MA Bioproducts), and secondary African Green monkey kidney (prepared from primary cultures, Flow Laboratories, McLean, VA) were grown and maintained at 36°C in Corning 75 cm^2 tissue culture flasks (Scientific Products, McGaw Park, IL) using E-MEM supplemented with 50 μg penicillin G, 50 μg streptomycin sulfate and 100 μg neomycin sulfate/ml (1% PSN, Gibco). 7–10% heat-inactivated fetal calf serum (HIFCS, MA Bioproducts) was added to the medium for cell growth (growth medium) and the concentration was reduced to 1–2% for cell maintenance (maintenance medium).

Virus CPE inhibition assays

Virus CPE inhibition assays were performed in HeLa or MRC-5 cells according to

the methods described previously [30]. A standard control compound, 6-(4-nitrophenoxy)-3-pyridinecarbonitrile, was also run in each assay. Control compound MIC_{50} values for RV-1A, RV-2 and Cox A21 ranged from 3.1 to 6.3 μg/ml for all assays.

Plaque assays

Plaque assays were performed as described previously [8] using HeLa cells and an overlay composed of 87% (v/v) McCoy's 5a medium supplemented with 2% HIFCS, 1% PSN, 10% of a 3.5% Ionagar (Oxoid U.S.A., Inc., Columbia, MD) solution, 2% of a 1.5 M solution of $MgCl_2 \cdot 6H_2O$, and 1% of a 0.2% dextran solution (DEAE-dextran, MW 2×10^6, Pharmacia, Uppsala, Sweden).

Compound preparation for mouse tests

For the single oral dose test, compounds were suspended in 0.5% aqueous hydroxypropyl methylcellulose (Methocel® type MC Premium, vis. 15 cps., The Dow Chemical Company, 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 preparation of 0.0038–0.06% compound-containing food 0.038–0.6 g of each compound was dissolved in 0.4–6.0 ml acetone and then mixed with 0.4–6.0 g silica gel (Hi-Sil 233, P.P.G. Inds., Pittsburgh, PA). After acetone evaporation, the gel–compound complex was admixed with 1.0 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 4.7–75.0 mg/kg mouse weight [10]. Compound-free control food was prepared in a similar manner.

Mouse tests

For the single dose antiviral tests, mice (9–12 g random bred Swiss albino, Harlan Sprague–Dawley, Inc., Indianapolis, IN) were challenged i.p. with 0.2 ml Cox A21 3 h before administration of the compound as an oral gavage. In the continuous dose test, animals were placed on compound-containing or placebo food 24 h before i.p. Cox A21 challenge. Compound-containing or placebo food was then continued ad libitum throughout the test period. For such assays, the virus was diluted in phosphate-buffered saline (PBS, pH 7.2 ± 0.1) or Hank's balanced salt solution (Gibco) containing 1% HIFCS to a concentration sufficient to cause 57–100% mortality of infected control mice within 9 days of an i.p. injection of 0.2 ml virus. All mice were observed for 8 to 9 days for deaths. A modified Mantel-Haenszel combined chi-square analysis [13] was then used to determine the difference between treated and placebo groups. This analysis incorporates both day of death and number of survivors into a single variable to measure the effectiveness of treatment. All compounds were initially screened in the single oral dose and feed tests at one concentration. The best compound was then re-evaluated in dose response assays. In these assays groups of 15 to 40 mice were used for each test dose.

Mechanism of compound action

The effects of test compound on viral (actinomycin D resistant) and cellular RNA synthesis were measured indirectly by determining incorporation of [5-^{3}H]uridine into a trichloroacetic acid (TCA)-insoluble cell fraction. The effects of test compound on cellular DNA and protein synthesis were measured indirectly by the incorporation of [6-^{3}H]thymidine and a L-[U-^{14}C] amino acid mixture respectively into a TCA-insoluble cell fraction. Virion uncoating was monitored using neutral red photosensitized Cox A21. Detailed descriptions of these techniques have been published previously [8,9,30].

Results

Preliminary screening for in vitro and in vivo anti-picornavirus activity and for cell toxicity

Fifteen *p*-benzoylphenoxypyridines were tested for their in vitro activity against RV-1A, 2 and 64 and against Cox A21 (Table 1). In addition, all of these compounds were evaluated for their in vivo therapeutic (single oral dose test) and prophylactic (continuous oral dose or feed test) efficacy in mice lethally infected with Cox A21 (Table 2). These studies showed the *p*-benzoylphenoxypyridines are relatively devoid of anti-RV activity (MIC_{50} of <3.1 to $>$ 10 μg/ml). In contrast, most of these compounds exhibit high Cox A21 activity. The best in vitro Cox A21 activity ($MIC_{50}<3.1$ μg/ml) is observed in those compounds possessing a 5-substituted methylsulfonyl (compound 1); ethylsulfonyl (compound 6); phenylmethylsulfonyl (compound 3); phenylsulfone (compound 4); nitro (compound 7); ethylthio (compound 9) or chloro (compound 10); or, a 3-cyano (compound 11) in the pyridine ring. Seven of these eight compounds are significantly active in vivo (Table 2). Of these, only compounds 1 and 6 exhibit highly significant ($P<0.001$) in vivo anti-Cox A21 activity in both the single oral dose and in the feed test (Table 2). The remaining 7 compounds do not show significant in vivo activity (data not shown).

Evaluation of the *p*-benzoylphenoxypyridines for HeLa cell toxicity (Table 1) shows that compound 2 is cytotoxic at a concentration of 5.0 μg/ml. All other compounds evaluated are devoid of HeLa cytotoxicity up to concentrations of 10 μg/ml.

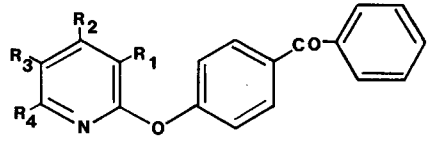
Expanded spectrum of activity studies

The results of the initial screening experiments led to expanded studies on the activities of compound 1 {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone and a comparison with its 3,4-dichloro analogue 2-(3,4-dichlorophenoxy)-5-methylsulfonyl pyridine [14] (Fig. 1). In vitro studies showed compound 1 to have less overall anti-RV activity than its 3,4-dichloro analogue (Table 3). In contrast, compound 1 is 4–70-fold more active against 6 of 12 (50.0%) of the enteroviruses (Cox A7, A9, A21 and B3; echoviruses 6 and 30) studied (Table 4). No differences in in vitro activity were noted for Cox B4, poliovirus 2, echoviruses 12 and 19, and enteroviruses 70 and 71.

The in vivo activity of compound 1 was re-evaluated in dose–response studies (Table 5). This compound is significantly active ($P<0.02$) in the single dose test at concentra-

TABLE 1

In vitro anti-picornavirus activity of some *p*-benzoylphenoxypyridines



Comp.	R_1	R_2	R_3	R_4	Lowest concentration (μg/ml) that caused				
					Cyto-toxicity	⩾50% reduction in viral CPE			
						RV-1A	RV-2	RV-64	Cox A21
1	H	H	$-SO_2CH_3$	H	> 10	> 10	5.0	2.5	1.3
2	H	H	$-SO_2CH(CH_3)_2$	H	5.0	> 5.0	> 5.0	> 5.0	> 5.0
3	H	H	$-SO_2CH_2$-phenyl	H	> 10	> 10	> 10	> 10	⩽ 3.1
4	H	H	$-SO_2$-phenyl	H	> 10	3.1	> 10	> 10	⩽ 3.1
5	H	H	$-SO_2(CH_2)_5CH_3$	H	> 10	3.1	> 10	> 10	3.1
6	H	H	$-SO_2CH_2CH_3$	H	> 10	< 3.1	6.3	6.3	1.3
7	H	H	$-NO_2$	H	> 10	> 10	3.1	> 10	1.3
8	H	H	$-NH_2$	H	> 10	3.1	3.1	> 10	3.1
9	H	H	$-SCH_2CH_3$	-CN	> 10	> 10	> 3.1	> 10	< 0.2
10	H	H	Cl	-CN	> 10	3.1	6.3	> 10	0.6
11	-CN	H	H	H	> 10	> 10	> 10	> 10	⩽ 3.1
12	$-SO_2CH_3$	H	H	H	> 10	> 10	> 10	> 10	> 10
13	$-SO_2CH_2CH_3$	H	H	H	> 10	> 10	> 10	> 10	> 10
14	H	H	H	$-SO_2CH_3$	> 10	6.3	6.3	> 10	6.3
15	H	-CN	H	H	> 10	6.3	3.1	3.1	> 10

TABLE 2

In vivo activity of some *p*-benzoylphenoxypyridines in the single oral dose and feed tests

Test	Compound[a]	Dose[b]	Test day						Combined χ^{2d}	Significance (*P*)
			4	5	6	7	8	9		
Single oral dose[e]	1	600	30/30 : 29/30[c]	30/30 : 27/30	26/30 : 19/30	26/30 : 17/30	24/30 : 11/30	22/30 : 10/30	19.38	<0.001
	4	600	30/30 : 29/30	21/30 : 26/30	19/30 : 15/30	16/30 : 7/30	10/30 : 1/30	10/30 : 1/30	5.80	<0.02
	6	600	30/30 : 30/30	29/30 : 23/30	29/30 : 11/30	28/30 : 3/30	27/30 : 2/30	27/30 : 2/30	45.21	<0.001
	7	600	30/30 : 27/29	30/30 : 24/29	27/30 : 18/29	20/30 : 5/29	16/30 : 4/29	16/30 : 4/29	14.18	<0.001
	9	400	19/30 : 20/30	17/30 : 19/30	15/30 : 12/30	12/30 : 8/30	9/30 : 3/30	9/30 : 3/30	5.73	<0.02
Feed[f]	1	75	15/15 : 9/11	15/15 : 7/11	15/15 : 6/11	15/15 : 3/11	15/15 : 1/11	15/15 : 1/11	24.39	<0.001
	2	75	14/15 : 12/14	14/15 : 6/14	12/15 : 1/14	10/15 : 0/14			17.70	<0.001
	6	75	15/15 : 12/15	15/15 : 9/15	15/15 : 6/15	15/15 : 4/15	15/15 : 3/15	15/15 : 3/15	20.44	<0.001
	9	75	28/29 : 30/30	28/29 : 23/30	28/29 : 19/30	28/29 : 12/30	28/29 : 11/30	28/29 : 11/30	27.47	<0.001
	11	75	14/15 : 8/15	8/15 : 3/15	3/15 : 1/15	0/15 : 0/15			4.91	<0.05

[a] From Table 1.

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

[c] Survivors/total for test compound: Survivors/total for placebo.

[d] Based on modified Mantel-Haenszel combined chi square analysis with 1 degree of freedom.

[e] Dose expressed in mg/kg.

[f] Dose expressed in mg/kg per day.

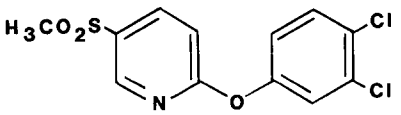


Fig. 1. Structure of 2-(3,4-dichlorophenoxy)-5-(methylsulfonyl)pyridine.

tions as low as 37.5 mg/kg. In comparison, its 3,4-dichloro analogue (Fig. 1) is significantly active ($P < 0.001$) at a low dose of only 150 mg/kg. In the continuous dose test, compound 1 was significantly active ($P < 0.001$) at a daily dose as low as 4.8 mg/kg while its 3,4-dichloro analogue was active ($P < 0.001$) in a similar test at a dose of as low as 37.5 mg/kg per day.

Mechanism of antiviral action

Mechanism of action studies showed that compound 1 inhibits coxsackievirus A21 (actinomycin D resistant) RNA synthesis by 50% and 95% at concentrations of 0.13 and 0.78 μg/ml, respectively (Fig. 2). As a result, effects of compound on earlier events in the virus replicative cycle were studied.

Studies by McSharry et al. [17] and by Mandel [12] have shown that incorporation of neutral red by picornaviruses during replication renders the progeny virions sensi-

TABLE 3

Expanded spectrum of anti-rhinovirus activity of { 4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl } phenyl methanone (compound 1) and its 3,4-dichloro analogue

		Compound 1	3,4-dichloro analogue
Rhinovirus	1A	> 10.0	0.3
	1B	> 5.0	< 0.3
	2	5.0	0.6
	4	3.1	> 10.0
	5	10.0	> 10.0
	8	> 10.0	> 10.0
	9	5.0	10.0
	10	10.0	1.3
	13	> 5.0	> 5.0
	21	> 10.0	10.0
	29	> 5.0	1.3
	32	5.0	< 1.3
	33	> 5.0	0.6
	39	> 10.0	6.3
	44	> 5.0	5.0
	64	2.5	0.3
	68	> 10.0	> 10.0
	74	> 10.0	2.5
	89	5.0	1.3
	Hank's	10.0	10.0

The data are expressed as the MIC_{50} in μg/ml.

TABLE 4

Expanded spectrum of enterovirus activity of {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone (compound 1) and its 3,4-dichloro analogue

		Compound 1	3,4-dichloro analogue
Coxsackievirus	A7[a]	0.3	5.0
	A9[b]	0.3	10.0
	A21[a]	1.3	6.3
	B3[a]	<0.6	>10.0
	B4[a]	10.0	>10.0
Echovirus	6[a]	<0.06	0.3
	12[a]	0.01	<1.3
	19[b]	<0.6	0.6
	30[a]	<0.6	2.5
Poliovirus	2[a]	>10.0	>10.0
Enterovirus	70[a]	2.5	2.5
	71[a]	5.0	>10.0

The data are expressed as the MIC_{50} in μg/ml.

[a] Assayed in HeLa cells.

[b] Assayed in MRC-5 cells.

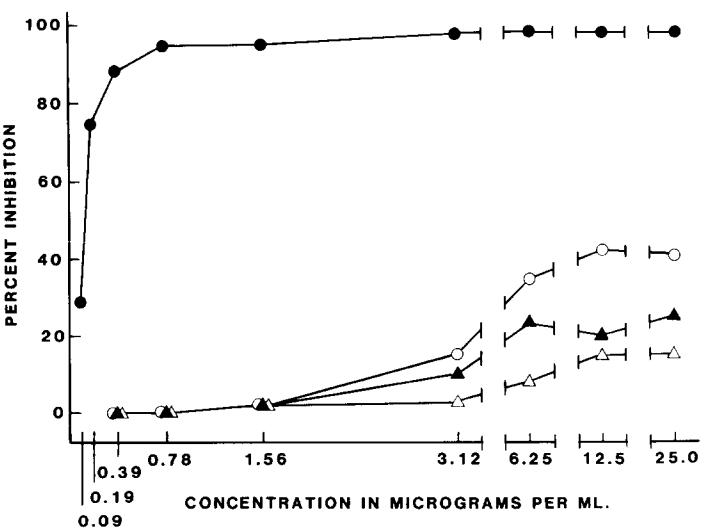


Fig. 2. Effect of {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone on coxsackievirus A21 RNA synthesis (●) and on HeLa cell DNA (▲), RNA (○) and protein (△) synthesis.

TABLE 5

In vivo comparative dose response of {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone (compound 1) and its 3,4-dichloro analogue administered as a single oral dose or in the food

Test	Compound	Dose	Test day						Combined χ^{2a}	Significance (*P*)
			4	5	6	7	8	9		
Single oral dose[d]	1	150	28/30[b]	27/30	25/30	24/30	21/30	21/30	23.95	<0.001
		75	29/30	27/30	21/30	18/30	17/30	17/30	10.97	<0.001
		37.5	29/30	26/30	20/30	16/30	12/30	12/30	5.59	<0.02
		18.8	30/30	28/30	17/30	13/30	8/30	8/30	1.79	N.A.[c]
		Placebo	29/30	25/30	17/30	7/30	4/30	4/30	-	-
	3,4-Di-	150	30/30	30/30	29/30	24/30	20/30	19/30	24.77	<0.001
	chloro	75	30/30	27/30	20/30	12/30	6/30	4/30	1.27	N.A.
	analogue	Placebo	28/30	27/30	14/30	6/30	5/30	3/30	-	-
Feed[e]	1	37.5	29/30	29/30	27/30	27/30	25/30	25/30	43.48	<0.001
		18.8	29/30	28/30	24/30	20/30	18/30	18/30	23.05	<0.001
		9.4	25/30	22/30	17/30	11/30	11/30	11/30	5.65	<0.02
		4.7	29/30	24/30	20/30	18/30	13/30	13/30	12.59	<0.001
		Placebo	27/30	19/30	10/30	3/30	2/30	2/30	-	-
	3,4-Di-	37.5	40/40	36/40	30/40	24/40	21/40	21/40	11.17	<0.001
	chloro	18.8	37/40	29/40	23/40	19/40	14/40	14/40	1.97	N.A.
	analogue	Placebo	39/40	34/40	17/40	11/40	7/40	7/40	-	-

[a] Based on modified Mantel-Haenszel combined chi square analysis with 1 degree of freedom.

[b] Survivors/total.

[c] Not active.

[d] Dose expressed as mg/kg.

[e] Dose expressed as mg/kg per day.

TABLE 6

Effect of {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone (compound 1) on the uncoating of coxsackievirus A21

Test condition	Infected HeLa cells exposed to light (H post-infection)	Compound added[a]	Avg. No. of plaques[b]		Percent plaque reduction	
			Expt. 1	Expt. 2	Expt. 1	Expt. 2
A	0	Yes	0	0	-	-
B	0	No	0	0	-	-
C	3	Yes	6(14)[c]	6(9)[c]	94.2(86.4)[c]	95.7(93.5)[c]
D	3	No	103	139	-	-
E	No light	Yes	81	150	55.7	32.7
F	No light	No	183	223	-	-
G	No light	Yes (retained)	0	0	100	100

[a] Compound present at 10 μg/ml through periods of virus adsorption and light inactivation. All plates were then washed three times (except condition G) and agar overlay medium added.

[b] Average number of plaques in three replicate cultures.

[c] Corrected for the residual compound effect demonstrated in a comparison of conditions E and F. Thus, 6 and 6 PFU represent only 44.3 and 67.3%, respectively of the number of plaques expected in the absence of a residual compound effect.

tive to light until the dye is lost after uncoating. Table 6 summarizes the effect of compound 1 on the uncoating of photosensitized coxsackievirus A21. In these studies HeLa cells were challenged with 183–223 PFU Cox A21 in the presence or absence of 10 μg compound 1/ml. One set of cultures (conditions A and B) was exposed to light within 5–10 min after addition of virus. A second set (conditions C and D) was exposed to light 3 h after virus challenge. The third set (conditions E through G) received no light exposure. All monolayers, except the compound controls (condition G), were thoroughly washed 3 h after virus challenge, agar overlay medium added and the plaques allowed to develop. The photosensitivity of the Cox A21 used in these studies is shown by the lack of plaque development in those cultures where light exposure was begun immediately after virus addition before uncoating could occur (conditions A and B). The amount of virus uncoated within 3 h of inoculation in untreated virus control cultures (condition D) was found to be 56.3–62.3% of the amount generated in cultures never exposed to light (condition F). Complete inhibition of plaque development was observed in unexposed cultures maintained on 10 μg compound 1/ml throughout the duration of the test (condition G). In these experiments not all test compound could be removed by washing as illustrated in a comparison of conditions E and F. Nevertheless, compound 1 reduced Cox A21 plaques by 86.4–93.5% in treated cultures exposed to light 3 h after inoculation when compared to non-treated cultures (conditions C vs. D).

Finally, exposure of RV-1A to 20 μg compound 1/ml maintenance medium for 4 h at 40–41°C has no effect on virus titer. In replicate experiments preincubation titers of

5.8 and 6.0 $\log_{10}$ $TCID_{50}$/ml and post incubation titers of 6.0 and 6.0 $\log_{10}$ $TCID_{50}$/ml were obtained, respectively. Taken collectively, these data suggest that compound 1 inhibits picornavirus uncoating or some earlier virus–host cell-associated event.

Compound effects on host cell metabolism

Isotopic studies (Fig. 2) show HeLa cell RNA, DNA and protein synthesis are inhibited by 41%, 25% and 13%, respectively, at 25 μg compound/ml.

Discussion

The picornaviruses are associated with a wide spectrum of human disease including encephalitis, aseptic meningitis, pericarditis, myocarditis, hepatitis and upper respiratory tract disease. Many compounds have shown promising in vitro and in vivo antipicornavirus activity including the guanidines [2,22,28]; the benzimidazoles [7,11,19,24–26,31]; the triazinoindoles [1,3,5,6,20,23,29]; and the isoquinolines [4]. Due to overt in vivo toxicity, low therapeutic ratios, or undesirable pharmacokinetic characteristics, few of these componds have been advanced to clinical studies and none have shown clinical efficacy in man. More recently, the arylalkyldiketone arildone and the isoxazole WIN 51711 have been shown to possess potent in vitro and in vivo anti-picornavirus activity [15,16,18]. Presently there are no published clinical evaluations of these two compounds.

In our efforts to develop orally active blood spectrum anti-picornavirus compounds we have shown that the phenoxybenzene, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile, is active in vitro against a large spectrum of picornaviruses at concentrations of 1.5 μg/ml or less in the CPE inhibition assay and that yields of progeny virions are reduced by > 1 $\log_{10}$ at compound concentrations of 1.0 μg/ml with 80% of the strains evaluated [21,30]. Those data also confirm the observations of Markley et al. [14] that good overall anti-picornavirus activity of the diaryl ethers requires electron deficient rings and that those compounds possessing electron withdrawing groups such as 2-cyano-4-nitro and 3,4-dichloro substitutions show the best activity. Concern over the potential toxicity associated with aromatic nitro compounds, however, led us to synthesize and evaluate additional diaryl ether analogues. Studies by Kenny et al. [8] on a series of 3,4-dichlorophenoxypyridinecarbonitriles have shown the overall anti-picornavirus activity of these compounds is reduced in comparison to 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile. In addition, systemic bioavailability is low after oral administration to mice. The present study shows that replacement of the 3,4-dichloro group on the benzene moiety by a *p*-benzoyl group also yields compounds with low RV activity. Several analogues of this series, however, demonstrate good in vitro and in vivo activity against Cox A21. The best compound, {4-[(5-methylsulfonyl-2-pyridinyl)oxy]phenyl} phenyl methanone (compound 1), was further compared with its 3,4-dichloro analogue. Expanded spectrum of activity studies further demonstrate that 3,4-dichloro replacement with a *p*-benzoyl substituent reduces RV activity but improves in vitro and in vivo enterovirus activity.

Compound 1 inhibits virion uncoating; a property shared with arildone [17], WIN

51711 [15] and the anti-RV 4',6-dichloroflavan BW683C [27]. Compound 1 also perturbs HeLa cell macromolecular synthesis at concentrations of as low as 3.12 μg/ml. This concentration is only 4-fold higher than the concentration of compound 1 necessary to inhibit Cox A21 RNA synthesis by 95%. This narrow therapeutic ratio limits the potential clinical utility of this compound to the most serious picornavirus infections.

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