Synthesis and Structure–Activity Studies of Some Disubstituted Phenylisoxazoles against Human Picornavirus

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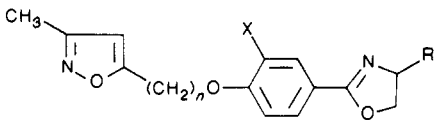
A number of 2,6-disubstituted analogues of disoxaril, a broad spectrum antipicornavirus agent, have been prepared and evaluated against several rhinovirus serotypes. A QSAR study revealed that the mean MIC ($\overline{MIC}$) against five rhinovirus serotypes correlated well with log P. The 2,6-dichloro analogue, **15**, was highly effective in vitro against rhinoviruses with an MIC_{80} of 0.3 μM, as well as against several enteroviruses, and was also effective in preventing paralysis in mice infected with coxsackievirus A-9.

Compounds of general structure **1** have been shown to exhibit broad spectrum antipicornavirus activity both in vitro[1-4] and in vivo.[5] Disoxaril (**1**, n = 7, X = H, R = H)



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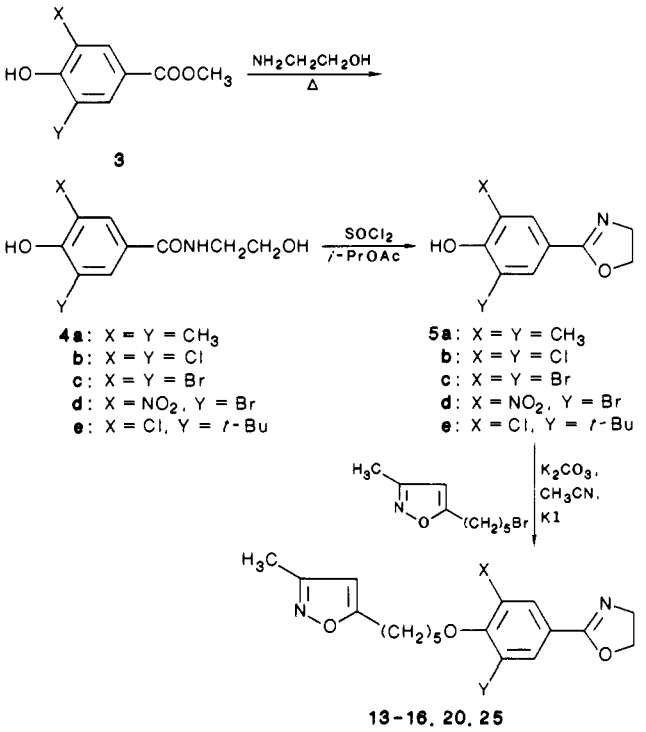
was found to be effective in vitro against most enteroviruses and rhinovirus serotypes tested. It was also orally effective in preventing poliovirus-2 and echovirus-9 induced paralysis in mice.[6] Previous studies have shown that the addition of substituents at the 2-position of the phenyl ring greatly improved antirhinovirus activity when compared to the unsubstituted analogues. In this series, a five-carbon chain was required for optimum activity. Furthermore, the mean MIC (minimum inhibitory concentration in a plaque reduction assay) of five serotypes selected to represent a range of sensitivity to this class of antiviral agent was found to be dependent upon log P, σ_m, and molecular weight (MW) as demonstrated by a regression analysis involving these physicochemical parameters.[4]

Mode of action studies have shown that disoxaril as well as other analogues in this series inhibit virus (rhinovirus type-2 and poliovirus type-2) replication by preventing viral uncoating.[7] Recently, X-ray crystallographic studies of disoxaril and of the 4-methyloxazoline analogue (**1**, n = 7, X = H, R = CH_3) bound to rhinovirus-14 clearly demonstrate that these compounds bind in a specific hydrophobic pocket within viral capsid protein 1 (VP 1).[8]

There are known to be approximately 100 human rhinovirus serotypes. A clinically useful broad spectrum antirhinovirus agent would therefore be required to inhibit a majority of these at a reasonable concentration. The situation is further complicated by the fact that each of these serotypes differ to varying degrees in the amino acid sequence of the viral capsid proteins. Since it is likely that binding of these compounds to the virion is dependent upon the amino acids in the binding pocket, any changes in this region could accordingly affect the binding and, consequently, the antiviral activity for a given compound.[9-13]

The monosubstituted phenyl compounds (**2**, X = H) exhibit a broader spectrum and greater degree of antirhinovirus activity than the unsubstituted analogues.[4] Consequently, we have prepared several 2,6-disubstituted

Scheme I. Method A



compounds **2** and have examined their antipicornavirus activity.

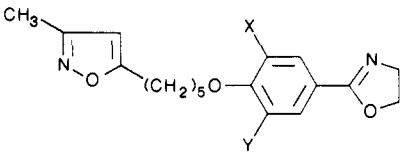


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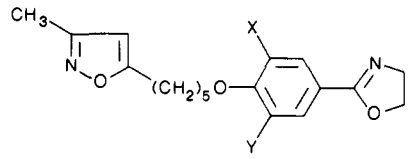
Table I. Antirhinovirus Activity



compd no.	X	Y	mp, °C	method	yield, %	formula[b]	in vitro activity:[a] MIC, μM mean[c]	MTL[d]
13	H	H	95–6	A	69	$C_{18}H_{22}N_2O_3$	5.16	9.9
14	CH_3	CH_3	*e*	A	45	$C_{20}H_{26}N_2O_3$	0.78	18.1
15	Cl	Cl	42–3	A	56	$C_{18}H_{20}Cl_2N_2O_3$	0.70	16.2
16	Br	Br	98–100	A	66	$C_{18}H_{20}Br_2N_2O_3$	0.63	13.1
17	F	F	49–50	C	28	$C_{18}H_{20}F_2N_2O_3$	2.69	35.7
18	Cl	CF_3	*e*	C	81	$C_{19}H_{20}ClF_3N_2O_3$	0.54	14.9
19	Cl	Br	41–2	C	57	$C_{18}H_{20}BrClN_2O_3$	0.61	29.2
20	NO_2	Br	*e*	A	7	$C_{18}H_{20}BrN_3O_5$	1.16	28.5
21	NO_2	Cl	*e*	B	51	$C_{18}H_{20}ClN_3O_5$	1.35	31.7
22	Cl	CH_3	*f*	C	74	$C_{19}H_{23}ClN_2O_3$	0.92	17.1
23	CH_3O	CH_3O	*e*	C	64	$C_{20}H_{26}N_2O_5$	9.9	33.4
24	CH_3	C_2H_5	*e*	B	83	$C_{21}H_{28}N_2O_3$	5.18	35.0
25	Cl	*t*-Bu	98–100	A	66	$C_{22}H_{29}ClN_2O_3$		12.4

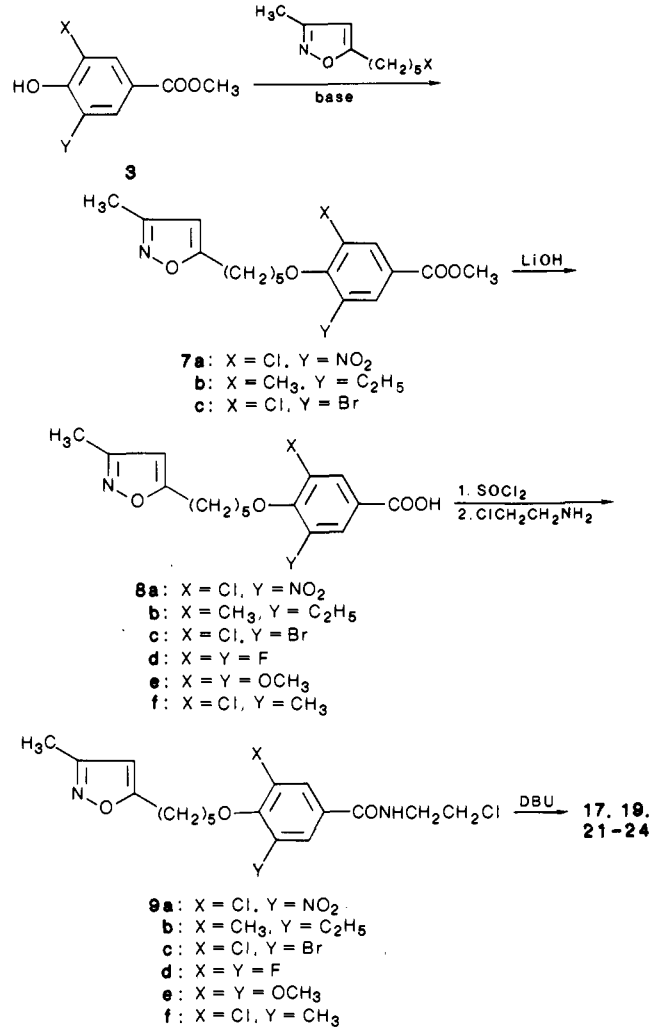
[a] Confidence limits p = 75%. [b] The elemental analyses (C, H, N) for all new compounds were within ±0.4% of the theoretical value. [c] Mean MIC for five serotypes, HRV-1A, -2, -22, -41, and -50. [d] Maximum testable levels (highest concentration of compound which causes no apparent effect on the cell monolayers). [e] Pure sample obtained by column chhromatography on silica gel by eluting with hexane–ethyl acetate (1:1). [f] Purified by HPLC, by using hexane–ethyl acetate (1:2).

Chemistry

Three methods of synthesis were employed depending upon the availability of starting materials. The methyl 4-hydroxybenzoates **3** were heated with ethanolamine to give the hydroxyethyl amides **4** (Scheme I). Treatment of **4** with thionyl chloride in isopropyl acetate gave oxazolines **5** in yields of 66–83%, which were then O-alkylated with 5-(5-bromopentyl)-3-methylisoxazole using potassium carbonate in acetonitrile, giving **6** in 27–50% yields.

An alternate synthesis that was used for the preparation of compounds **17**, **19**, and **21–24** is shown in Scheme II (method B). Ester **3** was converted to **7** by using either (5-bromo- or 5-hydroxypentyl)isoxazole[7] in ≃80% yield. In the latter case, diethyl azodicarboxylate and triphenylphosphine[19] were used as the coupling agents.

Scheme II. Method B



Esters **7** were hydrolyzed with LiOH to acids **8** in >90% yield. Treatment of the acids **8** with thionyl chloride followed by 2-chloroethylamine gave the chloroethyl am-

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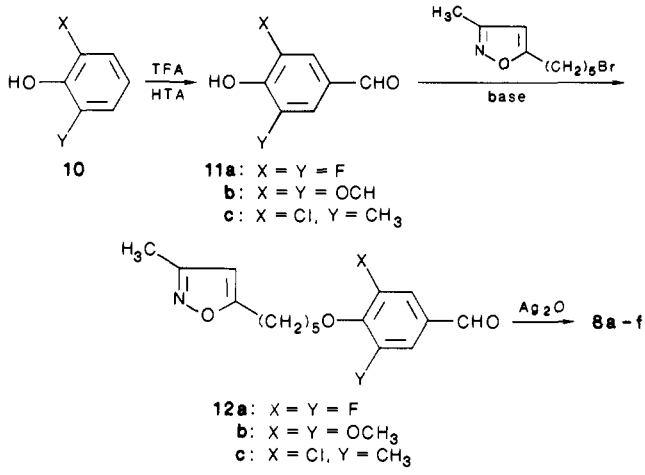
Table II. Expanded Spectra against 15 Rhinovirus Serotypes

compd no.	MIC against indicated rhinovirus serotype, μM															
	1A	1B	2	6	14	15	21	22	25	30	41	50	67	86	89	MIC_{80}
13	7.0	IA	1.1	0.06	0.7	1.5	1.2	0.9	IA	0.3	15.0	1.5	2.4	0.2	0.4	7.0
14	2.0	0.3	0.02	1.1	2.4	0.4	0.07	1.2	0.70	0.20	1.6	0.2	0.5	1.3	0.05	1.3
15	2.5	0.2	0.02	0.2	1.2	0.3	0.02	0.02	0.3	0.09	0.9	0.06	2.6	0.2	0.02	0.3
16	1.2	0.2	0.1	1.3	1.4	0.4	0.04	0.05	0.3	0.1	2.1	0.2	0.4	1.3	0.02	1.3
17	10.6	1.90	0.13	0.17	1.1	0.50	0.12	0.43	6.6	0.08	2.3	0.1	0.5	0.1	0.20	1.9
18	1.4	0.2	0.2	2.7	1.5	0.4	0.05	0.04	0.3	0.2	0.8	0.2	1.0	1.3	0.04	1.3
19	1.0	0.1	0.04	0.5	1.0	0.4	0.02	0.05	0.3	0.08	1.8	0.09	0.2	0.2	0.02	0.5
20	3.9	0.3	0.1	0.7	1.3	0.5	0.03	0.02	0.5	0.2	1.6	0.2	0.6	0.2	0.03	0.7
21	3.3	0.1	0.07	0.5	0.9	0.2	0.02	0.08	0.2	0.05	3.0	0.2	0.3	0.4	0.02	0.5
22	2.1	0.4	0.04	0.6	1.9	0.6	0.06	0.2	0.6	0.1	1.9	0.3	0.7	0.9	0.06	0.9
23	16.7	0.50	6.2	5.6	15.8	3.5	0.6	1.2	13.4	0.7	12.6	1.7	5.9	14.7	4.6	13.4

Scheme III. Method C



ides **9**, which were cyclized with DBU in methylene chloride with an overall yield for three steps of >50%.

The intermediates **8d–f** were prepared according to Scheme III (method C). Aldehydes **11**, prepared from phenols **10** by using the modified Duff reaction,[16] were O-alkylated with the (bromopentyl)isoxazole to give **12**. Treatment of the aldehyde **12** with Ag_2O gave acids **8d–f** in quantitative yield.

Biological Results

The compounds were initially screened against five rhinovirus serotypes (HRV-1A, -2, -22, -41, and -50) representing the range of sensitivities to this structural class of antiviral agent, and the mean MIC ($\overline{MIC}$) was determined. Eleven of the compounds tested (Table I) were effective against all five serotypes while the 2-ethyl-6-methyl (**24**) and 2-butyl-6-chloro (**25**) analogues were inactive against at least one serotype. Compounds that demonstrated no plaque reduction below the maximum testable level (MTL) were considered inactive.

Compounds **13** to **23** were evaluated against 10 additional rhinovirus serotypes, again representing a range of sensitivities. The results are shown in Table II. Compound **15** demonstrated the highest level of activity against the 15 serotypes tested with an MIC_{80} (concentration required to inhibit 80% of the serotypes tested in a plaque reduction assay) of 0.33 μM. Further evaluation of **15** against 45 serotypes resulted in an MIC_{80} of 0.58 μM with a range of MIC values of 0.02–2.6 μM. This compound was also effective against several enteroviruses, Table III. Echovirus-12 and coxsackievirus A-9 were particularly sensitive to compound **15** with MICs of 0.008 and 0.05 μM, respectively.

The antirhinovirus activity of compound **15** was compared to that of disoxaril in plaque reduction assays. Figure 1 shows the ranges of MIC values against 53 human

Table III. In Vitro Activity of Compound 15 against Enteroviruses

virus	MIC, μM
echovirus-3	0.18
-4	1.4
-6	0.09
-9	0.39
-9 (Barty)	0.44
-11	0.85
-12	0.008
-30	2.6
coxsackie A-2	8.6
A-4	19.8
A-8	0.03
A-9	0.03
A-10	13.2
A-11	16.2
A-16	4.8
A-21	0.07
B-1	0.21
B-2	0.08
B-3 (Gaunt)	0.13
B-4	0.29
B-5	0.34
poliovirus-1 (Mahoney)	1.4
-1 (Sabin)	0.65
-2 (MEF)	1.7
-2 (Lansing)	6.0
-3 (Leon)	0.55

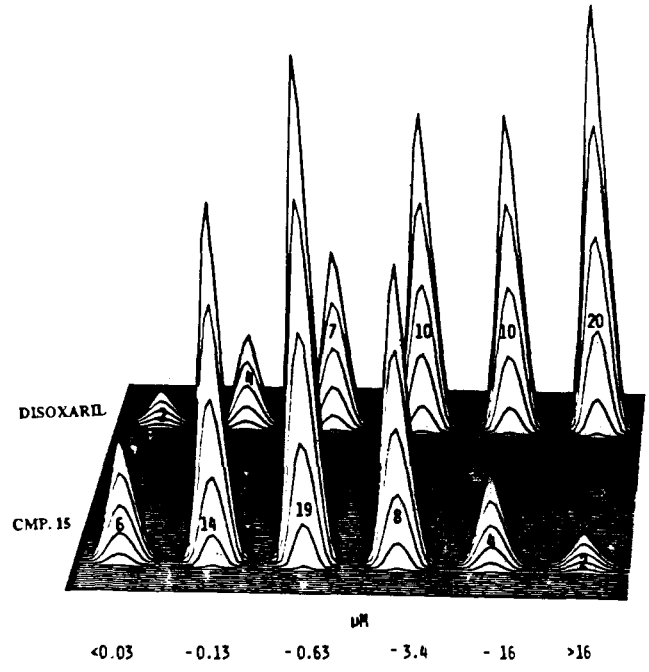


Figure 1. Comparative rhinovirus MIC distribution for disoxaril and compound **15** of 53 serotypes. Each peak represents the number of serotypes sensitive to the designated concentration of compound.

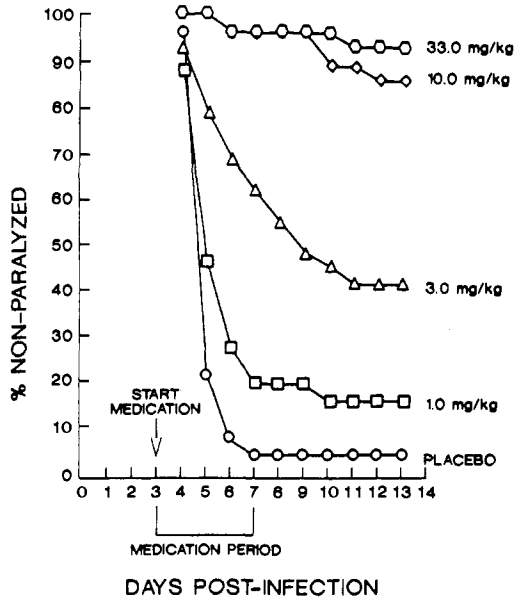


Figure 2. Effect of compound **15** in mice infected with coxsackievirus A-9. Mice were medicated once a day beginning 48 h post infection.

rhinovirus serotypes with each peak representing the number of serotypes sensitive to the two drugs in the specified ranges. The reduction in the size of the peaks at the higher levels for **15** indicates a broader spectrum of activity and greater virus sensitivity than disoxaril. Greater than 80% of the serotypes tested were inhibited by 0.25 μg/mL or less of **15** as compared to only 12% for disoxaril.

In Vivo Studies

Compound **15** was given orally in a single daily dose to suckling mice infected subcutaneously with coxsackievirus A-9 (see Experimental Section), beginning 48 h post infection and lasting for 5 days. The results are shown in Figure 2. This therapeutic regimen produced a dose-dependent protection from virus-induced paralysis with approximately 40% of the animals free of paralysis at doses as low as 3.0 mg/kg/day. Furthermore, after cessation of medication the number of mice developing paralysis did not continue to increase.

Quantitative Structure–Activity

In our analysis of the monosubstituted analogues in this series, we found a good correlation between $\overline{\text{MIC}}$ and molecular weight alone ($R = 0.81$).[4] There appeared to be a positive effect on activity with a concomitant increase in molecular weight. The addition of the log P term increased the correlation to $R = 0.88$. The introduction of σ_m as an electronic descriptor further improved the correlation to $R = 0.94$.

In the disubstituted series described here there was no correlation between $\overline{\text{MIC}}$ and σ_m or MW ($R = 0.30$ and 0.57, respectively). However, a good correlation was observed between log P and $\overline{\text{MIC}}$, $R = 0.93$ (eq 3, Table IV). A plot of log $[1/\overline{\text{MIC}}]$ vs log P (Figure 3) suggests a parabolic relationship in contrast to the linear relationship shown in eq 3. Despite the limited data set in this series, we examined the effect of incorporating σ_m and molecular weight in a regression analysis (eq 4, Table IV). Although there was a slight improvement in the correlation ($R = 0.96$), eq 4 shows a negative effect of molecular weight on activity. With a larger data set, the relationship would become more significant.

Table IV. Regression Analysis

no.	equation[a]	n	r	s
1	$\log [1/\overline{\text{MIC}}] = 3.06\ (\pm 0.405) \log P - 2.726\ (\pm 0.35)$	11	0.93	0.18
2	$\log [1/\overline{\text{MIC}}] = 0.49\ (\pm 0.37)\sigma_m - 0.386\ (\pm 0.23)$	11	0.40	0.44
3	$\log [1/\overline{\text{MIC}}] = 2.64\ (\pm 0.39)\text{MW} - 2.306\ (\pm 1.03)$	11	0.58	0.39
4	$\log [1/\overline{\text{MIC}}] = 3.28\ (\pm 0.53) \log P + 0.339\ (\pm 0.23)\sigma_m - 1.16\ (\pm 1.06)\text{MW} - 2.14\ (\pm 0.61)$	11	0.96	0.19

[a] Equations are normalized parameters; numbers in parentheses represent standard error of the estimate.

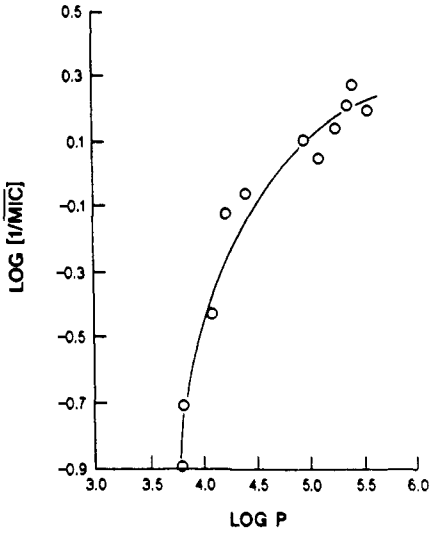


Figure 3. Plot of log $[1/\overline{\text{MIC}}]$ vs log P.

On the basis of the log P correlation alone, one would predict that compounds **24** (log $P = 4.90$) and **25** (log $P = 7.79$) would be very active. In actuality, **24** was weakly active and **25** was devoid of any activity, which again suggests a negative effect of bulk in the 2- and 6-positions, or a maximum in the correlation curve.

Using eq 3, values of $\overline{\text{MIC}}$ for compounds **13–25** were calculated and the results are shown in Table V. The calculated values for compounds **13–23** agree well with the measured $\overline{\text{MIC}}$. However, the values for **24** and **25** were not predictive.

Discussion

The results that were reported previously on the monosubstituted series[4] revealed that the antirhinovirus activity was dependent upon both (MW), lipophilicity (log P), and electronic effects σ_m. In this series, however, only log P appears to significantly affect activity while the bulk term produces a negative effect. This anomaly can be explained if the drug binding site in HRV-14 of disoxaril and the 4-methyloxazoline analogue is examined. These compounds bind in a hydrophobic pocket below the "canyon" which has been proposed as the cell receptor binding site.[8] In addition to the hydrophobic nature of this site, there are spatial constraints that would restrict the entry of compounds such as **24** and **25** and preclude them from exhibiting significant activity. Perhaps in the disubstituted series, the spatial limitations are exceeded by these compounds. The apparent parabolic curve generated by log $[1/\overline{\text{MIC}}]$ vs log P might be a reflection of these limitations. We commented in the introduction that differences exist among serotypes with respect to amino acid sequences in the vicinity of the drug-binding site. At this time, the crystal structure of only one rhinovirus

Table V. Physicochemical Constants

compd no.	X	Y	log P[a]	σ_m[b]	MW[c]	MIC, μM obsd[d]	MIC, μM cald[e]
13	H	H	3.83	0	0.665	5.2	4.05
14	CH_3	CH_3	4.95	−0.14	0.724	0.8	0.99
15	Cl	Cl	5.25	0.74	0.811	0.7	0.68
16	Br	Br	5.55	0.78	1.000	0.6	0.46
17	F	F	4.11	0.68	0.741	2.7	2.85
18	Cl	CF_3	5.42	0.80	0.883	0.5	0.55
19	Cl	Br	5.40	0.76	0.906	0.6	0.56
20	NO2	Br	4.41	1.10	0.928	1.2	1.95
21	NO_2	Cl	4.26	1.08	0.835	1.4	2.36
22	Cl	CH_3	5.10	0.30	0.769	0.7	0.82
23	CH_3O	CH_3O	3.79	0.24	0.792	7.7	4.27

[a] Estimated by using π[21] in combination with log P of compound 13 determined via RPHPLC – retention time procedure,[20] n = 3, mobile phase pH 7.4. [b] See ref 21. [c] Normalized to compound 16. [d] See Table I. [e] Calculated from eq 3.

Table VI. Parameter Correlation Matrix

	log P	σ_m	MW
log P	1.000		
σ_m	0.314	1.000	
MW	0.620	0.747	1.000

serotype (HRV-14) has been elucidated. However, the results of this study suggest that, at least for those serotypes for which amino acid sequences are known, the drug-binding pockets are also hydrophobic in nature since the lipophilicity of these compounds still correlates with their antiviral activity.

Experimental Section

Melting points were determined according to the USP procedure and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values. Analyses were performed by Galbraith Laboratories, Knoxville, TN. NMR spectra were determined on a JOEL FX-270 spectrophotometer and the mass spectra on a Jeolco double-focusing high-resolution mass spectrophotometer by Dr. S. Clemans.

In Vivo Test: Mouse Infection with Coxsackie A-9 Virus. Swiss albino suckling mice pups less than 24 h old weighing between 1.2 and 1.8 g were infected subcutaneously in the intrascapular region with 2.0 LD_{50}'s of coxsackievirus A-9. The mice were medicated orally once a day for 5 days beginning 2 days after infection at doses of 1, 3, 10, and 33 mg/kg of compound **15**. The animals were checked twice daily for evidence of paralysis. Paralyzed animals were noted and a tally kept of mice alive and not paralyzed for 13 days postinfection.

General Method of Synthesis. Method A. 4-Hydroxy-*N*-(2-hydroxyethyl)-3,5-dimethylbenzamide (4a). A mixture of 4.3 g (0.024 mol) of methyl 3,5-dimethyl-4-hydroxybenzoic acid[15] and 3.1 g (0.05 mol) of ethanolamine was heated to 160–165 °C for 2 h. After cooling, 10 mL of H_2O was added and the solution was acidified with 3 N HCl while cooling. An oil separated, which solidified; the resulting solid was collected by filtration and recrystallized from CH_3CN to give 3.2 g (68%) of **4a**. Anal. ($C_{11}H_{15}NO_3$) C, H, N. ^{1}H NMR: δ 8.0 (1 H, m, NH), 4.6 (1 H, m, OH), 3.5 (2 H, m, CH_2O), 3.3 (2 H, m, -$COCH_2$-) 2.2 (6 H, s, CH_3Ar × 2).

4-(4,5-Dihydro-2-oxazolyl)-2,6-dimethylphenol (5a). **4a** (14.4 g, 0.07 mol) was suspended in 200 mL of *i*-PrOAc, and 16.7 g (0.14 mol) of $SOCl_2$ in 50 mL of *i*-PrOAc was added dropwise. After the addition was complete, the mixture was stirred at room temperature for 18 h. The solid was collected and recrystallized from MeOH to give 10.6 g (67%) of **5a**, mp 208–210 °C. Anal. ($C_{11}H_{13}NO_2$·HCl) C, H, N. ^{1}H NMR: δ 3.7 (2 H, t, OCH_2), 3.5 (2 H, q, NCH_2).

5-[5-[4-(4,5-Dihydro-2-oxazolyl)-2,6-dimethylphenoxy]-pentyl]-3-methylisoxazole (14). A suspension of 13.8 g (0.1 mol) of K_2CO_3 and 9.0 g (0.06 mol) of NaI in 150 mL of DMF containing 6.8 g (0.03 mol) of **5a** and 13.9 g (0.06 mol) of 5-(5-bromopentyl)-3-methylisoxazole[16] was heated to reflux for 48 h under N_2. After cooling, the solids were separated by filtration and the filtrate was concentrated to dryness. The residue was partitioned between $CHCl_3$ and H_2O, the organic layer was dried, and the solvent was removed. The resulting oil was purified by MPLC by eluting with EtOAc/hexane (9:1). **14** (4.6 g, 45%) was obtained as an oil. Anal. ($C_{20}H_{26}N_2O_3$) C, H, N. ^{1}H NMR: δ 7.6 (2 H, s, Ar), 5.8 (1 H, s, =CH—), 4.4 (2 H, t, NCH_2), 3.8 (2 H, t, $ArOCH_2$), 2.25 (9 H, d, CH_3 × 3), 1.5–2.0 (6 H, m, CH_2 × 3).

Method B. Methyl 3-Bromo-5-chloro-4-hydroxybenzoate (3c). To a suspension of 5.0 g (0.028 mol) of 3-chloro-4-hydroxybenzoic acid in 75 mL of glacial acetic acid was added dropwise with stirring 4.5 g (0.028 mol) of bromine. After 24 h, the reaction was not complete (TLC) and an additional 3.1 g (.02 mol) of bromine was introduced. After an additional 24 h the solvent was removed from the resulting solution and the residual solid was dissolved in EtOAc, washed with water, and dried. Removal of the EtOAc in vacuo gave a white solid; 6.3 g (89%), mp 255–256 °C. This material was converted to its methyl ester with CH_3OH–HCl in 94% yield, mp 120–121 °C. Anal. ($C_8H_6BrClO_3$) C, H, N.

3-Bromo-5-chloro-4-[[5-(3-methyl-5-isoxazolyl)pentyl]-oxy]benzoic Acid (8c). The methyl ester **7c** was prepared from 41.3 g (0.16 mol) of **3c**, 61.7 g (0.45 mol) of K_2CO_3, and 40.0 g (0.18 mol) of 5-(5-bromopentyl)-3-methylisoxazole in 72% yield, after purification by MPLC, eluting with 1:8 EtOAc/hexane. Anal. ($C_{17}H_{19}ClBrNO_4$) C, H, N.

7c was hydrolyzed to the acid **8c** with LiOH in refluxing CH_3OH and H_2O in 86% yield after recrystallization from Et_2O/hexane, mp 104.5–105 °C. Anal. ($C_{16}H_{17}BrClNO_4$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 10.0 (1 H, s, COOH), 8.20 (1 H, d, J = 2 Hz, Ar), 8.08 (1 H, d, J = Hz, Ar), 5.85 (1 H, s, =CH—), 4.09 (2 H, t, J = 7 Hz, CH_2), 2.77 (2 H, t, J = 6 Hz, CH_2), 2.28 (3 H, s, CH_3), 1.6–2.0 (6 H, m, 3 CH_2).

3-Bromo-5-chloro-*N*-(2-chloroethyl)-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]benzamide (9c). A solution of 29.7 g (0.074 mol) of 3-bromo-5-chloro-4-[[5-(3-methyl-5-isoxazolyl)-pentyl]oxy]benzoic acid (**8c**) in 35 mL of $SOCl_2$ was stirred at room temperature for 16 h. The excess $SOCl_2$ was removed in vacuo and the residual oil dissolved in 250 mL of $CHCl_3$. To this solution was added 21 g (0.16 mol) of chloroethylamine hydrochloride. The mixture was cooled in an ice bath and treated with 80 mL (0.57 mol) of Et_3N. The mixture was stirred for 16 h at room temperature, then diluted with an additional 250 mL of $CHCl_3$, and washed with 2 × 250 mL of 1 N HCl. The organic layer was then dried and the solvent removed. The oil remaining was purified by column chromatography on silica gel, eluting with EtOAc/hexane (2:1), and after recrystallization from *i*-PrOH gave 30.9 g of **9c** (90%), mp 84–86 °C. Anal. ($C_{18}H_{21}BrCl_2N_2O_3$) C, H, N. ^{1}H NMR ($CDCl_3$): δ, 6.7 (1 H, br t, NHCO).

5-[5-[2-Bromo-6-chloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]pentyl]-3-methylisoxazole (19). A solution of **9c** (19 g, 0.041 mol) and 8 g (0.053 mol) of DBU in 250 mL of CH_2Cl_2 was heated to reflux for 24 h. The solvent was removed and the residual oil purified by MPLC, eluting with 1:1 EtOAc/hexane. After recrystallization from Et_2O, 10 g of **19** (57%) was obtained, mp 41–42 °C. Anal. ($C_{18}H_{20}BrClN_2O_3$) C, H, N. ^{1}H NMR ($CDCl_3$): δ 4.0–4.1 (4 H, m, 2 CH_2O), 4.44 (2 H, t, J = 9 Hz, CH_2).

3-Methyl-5-(5-hydroxypentyl)isoxazole. A suspension of 161 g of Amberlyst A-26 (carbonate form)[22] in 500 mL of benzene

and 50 g (0.215 mol) of 5-(5-bromopentyl)-3-methylisoxazole was heated to reflux for 4 h. The resin was removed by filtration and washed with CH_2Cl_2 and then MeOH and the filtrate was concentrated to dryness. The oily residue was distilled to give 20.8 g (57%) of the desired compound, bp 161–163 °C/12 mm. Anal. ($C_9H_{15}NO_2$) C, H, N.

Methyl 3-Chloro-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]-5-nitrobenzoate (7a). To a solution of methyl 3-chloro-4-hydroxy-5-nitrobenzoate (5.71 g, 0.025 mol), 4.44 g (0.020 mol) of 3-methyl-5-(5-hydroxypentyl)isoxazole, and 7.21 g (0.028 mol) of triphenylphosphine in 100 mL of CH_2Cl_2 was added under nitrogen 4.1 mL (0.020 mol) of diethyl azodicarboxylate in 25 mL of CH_2Cl_2 dropwise over a 2-h period.[17] The solution was stirred at room temperature overnight and then concentrated to dryness. The crude product was purified by column chromatography on silica gel and eluted with CH_2Cl_2 to give 8.3 g. Compound **7a** was obtained as a white solid in 78% yield, after recrystallization from EtOAc, mp 65–67 °C. Anal. ($C_{17}H_{19}ClN_2O_6$) C, H, N. ^1H NMR: δ 8.2, 8.3 (2 H, d, ArH X 2), 5.8 (1 H, s, =CH—), 4.2 (2 H, t, OCH_2), 3.9 (3 H, s, CO_2CH_3), 2.75 (2 H, t, $ArCH_2$), 2.3 (3 H, s, $ArCH_3$), 1.5–2.0 (6 H, m, CH_2 X 3).

3-Chloro-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]-5-nitrobenzoic Acid (8a). A solution of 35.1 g (.092 mol) of **7a**, 2.4 g (0.10 mol) of LiOH in 90 mL of CH_3OH, and 30 mL of H_2O was heated to reflux for 18 h. The solution was concentrated to dryness and the solid residue partitioned between EtOAc and dilute HCl. The organic phase was washed twice with H_2O and dried. Removal of the solvent gave a white solid, which was recrystallized from *i*-PrOAc–hexane to give 32.4 g (95%) of **8a**, mp 95–6 °C. Anal. ($C_{16}H_{17}ClN_2O_6$) C, H, N. ^1H NMR: δ 10.8 (1 H, br s, CO_2H).

Method C. 3-Chloro-4-hydroxy-5-methylbenzaldehyde (11c). To a solution of 154.5 g (1.08 mol) of 2-chloro-6-methylphenol in 1 L of trifluoroacetic acid was added portionwise at room temperature 152 g (1.08 mol) of hexamethylenetetramine.[18] An exothermic reaction took place. After the addition was complete, an additional 300 mL of trifluoroacetic acid was added in order to aid stirring. The suspension was heated at reflux overnight. The reaction was cooled, the excess TFA was removed in vacuo, and the resulting mixture was poured into 3 L of CH_2Cl_2. The organic layer was washed with water and then several times with 500-mL portions of 10% K_2CO_3 solution. The aqueous extracts were acidified with concentrated HCl and the resulting precipitate was collected. Recrystallization from EtOAc gave 115 g (62%) of **11c**, mp 114–116 °C. Anal. ($C_8H_7ClO_2$) C, H, Cl. ^1H NMR ($CDCl_3$): δ 9.8 (1 H, s, CHO), 7.7 (1 H, s, ArH), 7.6 (2 H, Br s, ArH, -OH), 2.3 (3 H, s, $ArCH_3$).

3-Chloro-5-methyl-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]benzaldehyde (12c). A mixture of 20 g (0.12 mol) of **11c**, 35 g (0.25 mol) of K_2CO_3, 28.0 g (0.12 mol) of 5-(5-bromopentyl)-3-methylisoxazole, and 100 mg of NaI in 300 mL of DMF was heated to 100 °C for 1.5 h. The cooled reaction mixture was filtered and the filtercake washed with DMF. Removal of the DMF from the filtrate gave an oily residue, which was partitioned between EtOAc and water. The organic layer was dried and concentrated to dryness. The resulting oil was purified by MPLC, eluting with EtOAc/hexane (1:4). Thirty-two grams (83%) of **12c** was obtained as a thick oil. Anal. ($C_{17}H_{20}ClNO_3$) C, H, N. ^1H NMR ($CDCl_3$): δ 9.9 (1 H, s, CHO), 7.7 (1 H, s, ArH) 7.6 (1 H, br s, ArH), 5.8 (1 H, s, H-isox), 4.0 (2 H, s, OCH_2-, J = 6 Hz), 2.8 (2 H, t, -CH_2-isox, J = 7 Hz), 2.4 (3 H, s, $ArCH_3$), 2.3 (3 H, s, isox CH_3), 1.9–1.6 (6 H, m, -$(CH)_2$).

3-Chloro-5-methyl-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]benzoic Acid (8f). To a stirred solution of 27.5 g (.086 mol) of **12c** in 150 mL of EtOH was added 34.1 g (.2 mol) of $AgNO_3$ in 50 mL of H_2O.[19] Stirring was continued for 15 min and then a 450-mL aqueous solution of 30 g (.54 mol) of KOH was added over a 50-min period. After stirring for an additional 50 min, the reaction mixture was filtered through Filtercel and the filtercake washed with H_2O. The filtrate was extracted with Et_2O and then acidified to pH 2 and extracted with EtOAc. The dried organic layer was concentrated to dryness to give 25.9 g (90%) of **8f**, mp 98–99 °C. Anal. ($C_{17}H_{20}ClNO_4$) C, H, N. ^1H NMR ($CDCl_3$): δ 10.8–10.3 (1 H, br s, COOH).

3-Chloro-*N*-(2-chloroethyl)-5-methyl-4-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]benzamide (9f). A solution of 59.2 g (.059 mol) of **8f** in 55 mL of $SOCl_2$ was stirred at room temperature for 18 h. The excess $SOCl_2$ was removed in vacuo and the residue dissolved in 300 mL of $CHCl_3$. To this solution was added 21.5 g (.185 mol) of 2-chloroethylamine hydrochloride and then, dropwise, 39 g of Et_3N. After the addition was complete, an additional 15 g of Et_3N was added and the mixture stirred for 18 h at room temperature. The mixture was washed with H_2O followed by 2 × 100 mL of 1 N HCl. The organic layer was dried and the solvent removed, leaving 25.2 g of a brown solid, which was recrystallized from Et_2O. **9f** (13.6 g, 57%) was obtained, mp 85–85 °C. Anal. ($C_{19}H_{24}Cl_2N_2O_3$) C, H, N. ^1H NMR: δ 6.6 (1 H, br s, NH), 3.9–3.7 (4 H, m, $Cl(CH_2)_2N$).

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(22) Cardillo, G.; Orena, M.; Dorzi, G.; Sandri, S. *Synthesis* **1981**, 793.