Table III. Physical Data for Synthetic Inhibitors

compd	yield,[a] %	mp,[b] °C	R_f[c]	$[\alpha]^{24}_D$,[d] deg	microanal.
23	67	238–239 (MeOH–Et_2O)	0.62	−51 (0.1, MeOH)	C, H, N
24	81	212–213 (MeOH–Et_2O)	0.43	−71 (0.1, MeOH)	C, H, N
25	54	279–280 (HOAc–Et_2O)	0.58	−95 (0.07, MeOH)	C, H, N
26	87	>250	0.21	−33 (0.21, MeOH)	C, H, N[e]
27	52	284 (HOAc–MeOH–Et_2O)	0.55	−36 (0.06, MeOH)	C, H, N
28	95	220–223	0.50	−64 (0.24, MeOH)	C, H, N
29	55	234–234.5 (MeOH–Et_2O)	0.49	−93 (0.07, MeOH)	C, H, N
32	70	227–228 (MeOH–Et_2O)	0.38	−93 (0.76, MeOH)	C, H, N
33	40	229–230 (MeOH–$CHCl_3$–hexane)	0.71	−80 (0.1, MeOH)	C, H, N[f]
34	83	238–239 (MeOH–Et_2O)	0.63	−21.5 (0.2, MeOH)	C, H, N[g]
35	51	252–244 (MeOH–$CHCl_3$–hexane)	0.62	−81 (0.08, MeOH)	C, H, N
36	81	222–223 (hexane)	0.57	−83 (0.54, MeOH)	C, H, N

[a] From acetoxy precursor **22**. [b] (Recrystallization solvent). [c] In 10% MeOH–$CHCl_3$. [d] (Concentration, solvent). [e] Calcd for $C_{34}H_{58}N_4O_5 \cdot H_2O$. [f] Calcd for $C_{28}H_{52}N_4O_5 \cdot 0.5MeOH$. [g] Calcd for $C_{34}H_{56}N_4O_5 \cdot 1.5MeOH$.

(5% MeOH–$CHCl_3$) R_f 0.33 (4*S*), 0.30 (4*R*). 270-MHz NMR of 4*S* isomer ($CDCl_3$): δ 0.6–1.7 (cmplx, 30 H) [includes δ 1.10 (d, J = 5.7 Hz, isostere C(2)-CH_3), 1.35 (d, J = 6 Hz, Ala-CH_3)], 1.85 (m, 2 H), 2.0–2.13 (cmplx, 7 H) [includes δ 2.10 (s, OAc)], 2.35 (m, 2 H), 3.25 (m, 2 H, Iaa α-CH_2), 4.2 (2 m, 2 H, Val-Hα and isostere C(5)-H), 4.4 (m, 1 H, Ala-H_2), 4.9 (m, 1 H, isostere C(4)-H), 5.95 (d, J = 8.5 Hz, 1 H), 6.17 (d, J = 10 Hz, 1 H), 6.3 (t, J = 6 Hz, Iaa-NH), 6.6 (d, J = 8.5 Hz, 1 H). Anal. ($C_{30}H_{56}N_4O_6$) C, H, N. NMR of 4*R* isomer ($CDCl_3$): δ 2.05 (s, OAc).

Preparation of Hydroxyethylene Peptides. An ca. 0.1 M solution of acetoxy peptide in MeOH was vigorously stirred with 10 molar equiv of anhydrous K_2CO_3 until the starting material was consumed (TLC, 5% MeOH–$CHCl_3$). Ordinarily 15–20 h of reaction time was allowed. Then 5–10 volumes of H_2O was added, the mixture was chilled, and the precipitated peptide was collected by filtration and washed with H_2O. Yields for crude product were usually >90%. Recrystallization from an appropriate solvent system (see Table III) afforded product used for characterization and for enzyme-inhibition studies. Physical data are collected in Table III. 360-MHz NMR (MeOH-d_4) of Iva-Val-Leu$\underline{^{OH}}$Ala-Ala-Iaa (**25**): δ 0.8–1.0 (cmplx, 24 H), 1.1 (d, J = 6.6 Hz, 3 H, isostere ((2)-CH_3), 1.3 (d, J = 6.6 Hz, 3 H, Ala-CH_3), 1.31–1.72 (cmplx, 7 H), 2.02 (m, 1 H, Val-β), 2.1 (m, 2 H, Iva-CH_2), 2.61 (m, 1 H, isostere C(2)-H), 3.18 (m, 2 H, Iaa α-CH_2), 3.68 (m, 1 H, isostere C(4)-H), 3.88 (m, 1 H, isostere C(5)-H), 4.1 (d, J = 7.8 Hz, 1 H, Val-Hα), 4.25 (m, 1 H, Ala-Hα).

Oxidation of the Hydroxyethylene Moiety to the Ketomethylene Moiety. Ordinarily, crude products from deacetylation of (*R,S*)-OAc precursors were used as the starting materials. To an ca. 0.02–0.04 M solution of starting hydroxyethylene peptide in glacial HOAc was added pyridinium dichromate (3 mmol/mol of peptide). The mixture was stirred until starting material was consumed (typically 5–25 h) and then diluted with equal volumes of $CHCl_3$ and H_2O. The layers were separated, and then the organic phase was subjected to standard workup. The product was purified by flash chromatography (ca. 200:1 w/w SiO_2–peptide), eluting with 1% MeOH–$CHCl_3$, followed by recrystallization from an appropriate solvent system. Physical data are collected in Tables III and IV. 360-MHz NMR (MeOH-d_4) for Iva-Val-Leu$\underline{^{K}}$Ala-Ala-Iaa (**33**): δ 0.8–1.0 (cmplx, 24 H), 1.1 (d, J = 6.6 Hz, 3 H, isostere C(2)-CH_3), 1.3 (d, J = 6.6 Hz, 3 H, Ala-CH_3), 1.32–1.78 (cmplx, 7 H), 2.02 (m, 1 H, Val-β), 2.1 (m, 2 H, Iva-CH_2), 2.57 (m, 1 H, isostere C(2)-H), 2.85 (m, 2 H, isostere C(3)-H), 3.18 (m, 2 H, Iaa α-CH_2), 4.2 (cmplx, 2 H, Ala-Hα and Val-Hα), 4.4 (m, 1 H, isostere C(5)-H).

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Structure-Activity Studies of 5-[[4-(4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]-3-methylisoxazoles: Inhibitors of Picornavirus Uncoating

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A series of substituted phenyl analogues of 5-[[4-(4,5-dihydro-2-oxazolyl)phenoxy]alkyl]-3-methylisoxazoles has been synthesized and evaluated in vitro against several human rhinovirus (HRV) serotypes. Substituents in the 2-position greatly enhanced activity when compared to the unsubstituted compound. Many of these compounds exhibited mean MICs ($\overline{MIC}$) against five serotypes as low as 0.40 μM. The mean MIC correlated well (r = 0.83) with the MIC_{80} (the concentration that inhibited 80% of the serotypes tested). A quantitative structure–activity relationship study indicated a strong dependency of $\overline{MIC}$ on lipophilicity (log P) in combination with inductive effects (σ_m) and bulk factors (MW).

Compound **1** is a broad-spectrum antipicornavirus agent[1] that inhibits replication of 36 out of 45 rhinovirus serotypes at levels ranging from 0.3 to 3.0 μM and is also effective against several enteroviruses.[2,3] When administered orally,[4] **1** reduces mortality in mice infected intracerebrally

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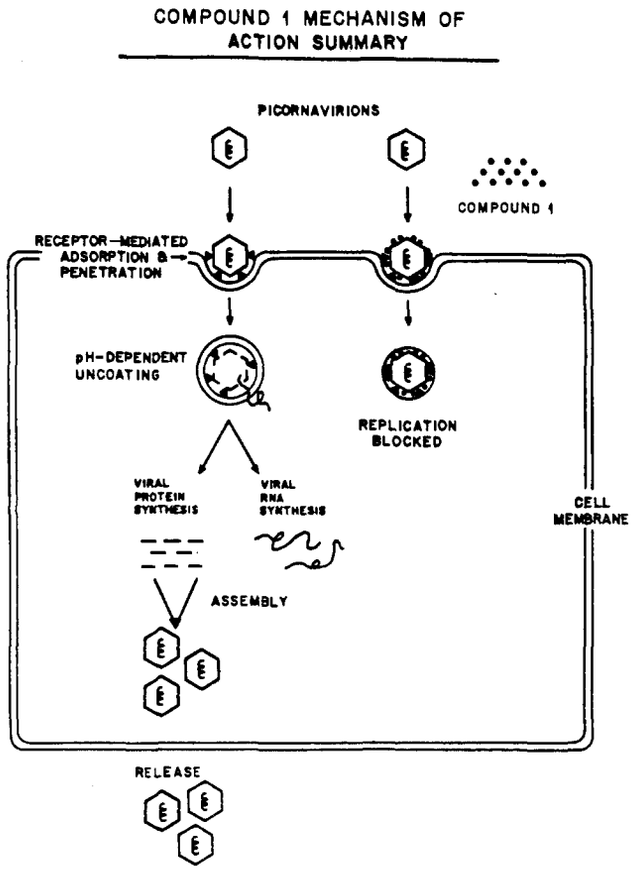
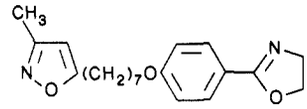


Figure 1.

with poliovirus-2 and also prevents paralysis when administered intraperitoneally to mice infected subcutaneously with Echo-9 virus.[5]

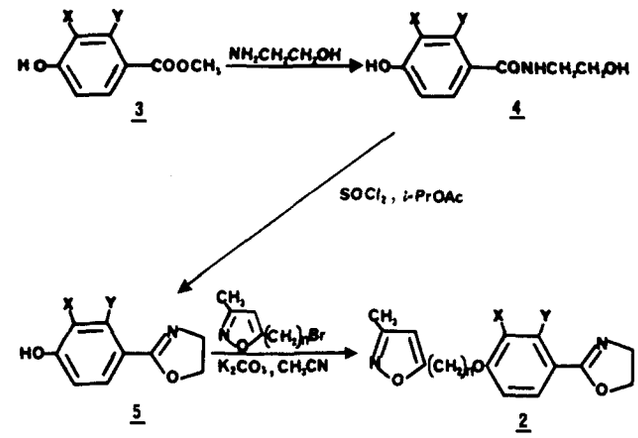


1

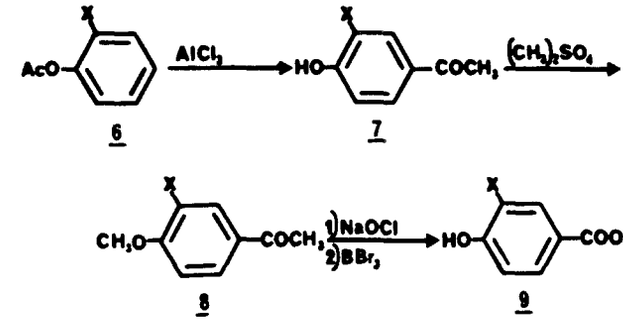
The mode of action of compound 1 has been shown to be the inhibition of viral uncoating.[6] The role of uncoating in the replication of picornaviruses is graphically shown in Figure 1. After receptor-mediated adsorption of the virus to the cell membrane, the virion penetrates the membrane and then uncoats, releasing the viral RNA. After transcription and translation, newly synthesized viral RNA is then inserted into a newly formed virion procapsid with subsequent release of the intact virus from the cell. Blocking viral replication by preventing uncoating of the virion represents a viable approach to antiviral chemotherapy.

Compound 1 is not unique in its ability to inhibit viral replication by preventing uncoating. Three unrelated compounds, 4′,6-dichloroflavan (BW-683C),[7-11] 4-ethoxy-2′-hydroxy-4′,6-dimethoxychalcone (RO 09-0410),[8,11,12] and 1-[5-(tetradecyloxy)-2-furanyl]ethanone (RM-15,731),[8,13] have been reported to exhibit varying levels of antirhinovirus activity by this mechanism.

Scheme I



Scheme II



Scheme III

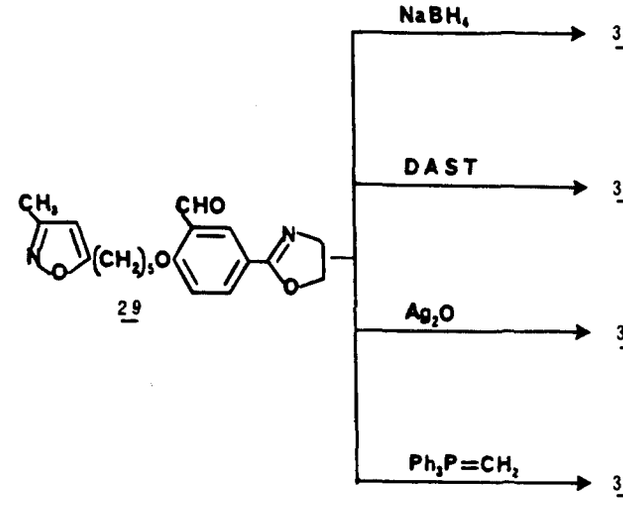


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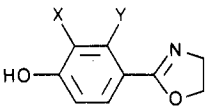
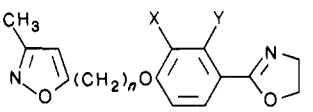
Table I. 4-(4,5-Dihydro-2-oxazolyl)phenol Intermediates



compd no.	X	Y	recrystn solvent	mp, °C	yield, %	formula
5a	Cl	H	*i*-PrOAc	156–157	99	$C_9H_8ClNO_2 \cdot HCl$
5b	F	H	THF	201–203	71	$C_9H_8FNO_2$
5c	Br	H	THF	200–201	54	$C_9H_8BrNO_2$
5d	NH_2	H	acetone	158–160	91	$C_9H_{10}N_2O_2$
5e	CH_3	H	MeOH	190–191	43	$C_{10}H_{11}NO_2$
5f	C_2H_5	H	MeOH	158–159	30	$C_{11}H_{13}NO_2 \cdot HCl$
5g	*i*-C_3H_7	H	EtOH	194–196	63	$C_{12}H_{15}NO_2$
5h	*t*-C_4H_9	H	MeCN/MeOH	243–245	71	$C_{13}H_{17}NO_2$
5i	NO_2	H	EtOH	125–126	70	$C_9H_8N_2O_4 \cdot HCl$
5j	CH_3O	H	*i*-PrOAc	184–185	51	$C_{10}H_{11}NO_3$
5k	CH_3CO	H	*i*-PrOAc	135–136	27	$C_{11}H_{11}NO_3$
5l	CHO	H	*i*-PrOAc	111–112	99	$C_{10}H_9NO_3 \cdot HCl$
5m	$N(CH_3)_2$	H	acetone	145–146	17	$C_{11}H_{14}N_2O_2$
5n	H	CH_3	MeOH	147–149	34	$C_{10}H_{11}NO_2$
5o	H	Cl	MeCN/MeOH	143–145	64	$C_9H_8ClNO_2$

In continuation of our interest in preventing rhinovirus uncoating as an approach to viral chemotherapy, we have prepared analogues of **1** with substituents in the 2- or 3-position, as shown in general structure **2**, and have examined their effect on antirhinovirus activity.



2

Chemistry

The majority of compounds were prepared by O-alkylation of a phenol **5** with the appropriate (bromoalkyl)-isoxazole according to Scheme I.[14] The hydroxyethyl amides **4** generally were not isolated but were converted directly to the oxazolines shown in Table I. O-Alkylation was performed with anhydrous potassium carbonate in either acetonitrile or DMF.

When the required 3-substituted 4-hydroxybenzoates **9** were not commercially available, they were prepared by a Fries rearrangement of the 2-substituted acetoxybenzenes **6** to give the acetophenones **7** in 50–80% yield[15] (Scheme II). Oxidation of the acetophenones, after protection of the phenol as the *O*-methyl ether, followed by demethylation and esterification gave the esters **9** in 40–60% yield from **7**. Compounds **30–32** and **36** were prepared from aldehyde **29** as shown in Scheme III.

Biological Results

Compounds were initially screened against human rhinovirus type-2 (HRV-2) in the plaque reduction assay previously described.[1] Those compounds demonstrating a MIC (minimum inhibitory concentration) of less than 3 μM against HRV-2 were further screened against four additional rhinovirus serotypes, HRV-1A, -22, -41, and -50, and the mean MIC ($\overline{\text{MIC}}$) was determined. Compounds inactive against HRV-2 were generally found to be ineffective against other rhinovirus serotypes. The screening results are shown in Table II. Compound **1**, as well as the five-carbon-chain homologue **10**, is included for comparison. Compounds with substituents in the position ortho to the ether were generally more active than the unsubstituted compounds. The 2-chloro analogue **14** was greater than 25 times more active than **1** against HRV-2 and had a significantly lower $\overline{\text{MIC}}$. When the length of the alkyl chain was varied, optimal activity was apparently achieved with the C_5 homologue **12** as measured by the $\overline{\text{MIC}}$ and activity against HRV-2. The 2-methyl analogue **15** was somewhat less active than compound **12**. The remaining variations were made with the C_5 alkyl chain.

Before proceeding to prepare other 2-substituted compounds, we examined the 3-chloro (**16**) and 3-methyl (**17**) analogues. Both of these compounds were considerably less active than the corresponding 2-chloro (**12**) and 2-methyl (**15**) analogues when tested against HRV-2 and as measured by $\overline{\text{MIC}}$.

Having established the importance of the 2-position with respect to activity, we prepared several additional analogues with substituents in this position. The two most active compounds were the bromo and trifluoromethyl analogues, **19** and **21**, respectively. The nitro analogue **25** was very potent against HRV-2 but had a higher $\overline{\text{MIC}}$ than **19** and **21**. Increasing the size of the 2-alkyl substituent to ethyl (**22**), isopropyl (**23**), or *tert*-butyl (**24**) resulted in diminished activity. Hydrophilic groups such as amino (**20**), hydroxymethyl (**30**), carboxyl (**32**), or dimethylamino (**35**) resulted in compounds with relatively weak activity.

Several of the more active compounds were screened against 10 additional rhinovirus serotypes, and the MIC_{80} (the concentration that inhibits 80% of the serotypes tested) was determined. The results are shown in Table III. Compound **10** was inactive against HRV-1B and -25, whereas the majority of the 2-substituted analogues shown in Table III were active against all 15 serotypes. Compounds **19** (2-Br) and **25** (2-NO_2) were the most effective and exhibited MICs in the range of 0.05–1.04 and 0.06–2.03 μM, respectively. Considerable differences were noted in sensitivity of the different serotypes to each of the compounds, which may be attributed to structural differences between each serotype.

Quantitative Structure–Activity Relationships

Since $\overline{\text{MIC}}$ was considered an early indicator of broad-spectrum activity, we examined its relationship to several physicochemical parameters. We had observed (Table II) that substituents in the 2-position exerted a substantial effect upon activity, depending upon the nature of these

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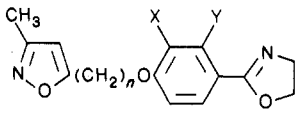
Table II. Antirhinovirus Activity of Monosubstituted Phenyloxazolines



compd no.	*n*	X	Y	mp, °C	yield, %	formula[b]	in vitro activity:[a] MIC, μM HRV-2[c]	mean[d]	MTL,[e] μM	MIC_{80}, μM
1	7	H	H	89–90	76	$C_{20}H_{26}N_2O_3$	3.5	>18	18	
10	5	H	H	95–96	69	$C_{18}H_{22}N_2O_3$	1.1	5.2	9.9	7.0
11	4	Cl	H	75–76	69	$C_{17}H_{19}ClN_2O_3$	0.20	1.0	9.3	3.6
12	5	Cl	H	102–103	65	$C_{18}H_{21}ClN_2O_3$	0.06	1.0	8.9	1.0
13	6	Cl	H	64–65	53	$C_{19}H_{23}ClN_2O_3$	0.06	1.4	8.5	1.2
14	7	Cl	H	120–121	73	$C_{20}H_{25}ClN_2O_3$	0.1	2.3	17	2.8
15	5	CH_3	H	90–91	58	$C_{19}H_{24}N_2O_3$	0.1	1.4	19	0.60
16	5	H	Cl	73–74	56	$C_{18}H_{21}ClN_2O_3$	0.40	6.5	25	7.0
17	5	H	CH_3	50–51	54	$C_{19}H_{24}N_2O_3$	0.41	>18	19	15.0
18	5	F	H	95–96	51	$C_{18}H_{21}FN_2O_3$	0.4	>9.3	9.3	
19	5	Br	H	123–124	42	$C_{18}H_{21}BrN_2O_3$	0.04	0.4	7.9	1.4
20	5	NH_2	H	131–132	7	$C_{18}H_{23}N_3O_3$	0.91	7.0	19	
21	5	CF_3	H	85–86	34	$C_{19}H_{21}F_3N_2O_3$	0.03	0.4	3.4	1.1
22	5	C_2H_5	H	83–85	65	$C_{20}H_{26}N_2O_3$	0.06	1.8	9.1	2.0
23	5	isopropyl	H	69–71	24	$C_{21}H_{28}N_2O_3$	0.2	>50	2.2	
24	5	*tert*-butyl	H	103–104	38	$C_{22}H_{30}N_2O_3$	IA[h]		8.4	
25	5	NO_2	H	94–95	24	$C_{18}H_{21}N_3O_5$	0.04	0.7	3.9	1.8
26	5	CH_3O	H	97–99	46	$C_{19}H_{24}N_2O_4$	0.3	4.5	37	1.9
27	5	$CH_2CH{=}CH_2$	H	68–69	52	$C_{21}H_{26}N_2O_3$	0.08	1.1	8.8	2.1
28	5	CH_3CO	H	117–118	31	$C_{20}H_{24}N_2O_4$	0.1	1.1	6.5	5.1
29	5	CHO	H	103–104	73	$C_{19}H_{22}N_2O_4$	1.4	>2.3	2.3	
30	5	CH_2OH[f]	H	140–141	99	$C_{19}H_{24}N_2O_4$	7.1		9.0	
31	5	CHF_2[f]	H	88–89	27	$C_{19}H_{22}F_2N_2O_3$	0.06	1.0	17	2.0
32	5	COOH[f]	H	170–171	37	$C_{19}H_{22}N_2O_5$	IA[h]			
34	5	$NHCOC_2H_5$[g]	H	133–134	44	$C_{21}H_{27}N_3O_4$	7.3		16	
35	5	$N(CH_3)_2$	H	80–81	19	$C_{20}H_{27}N_3O_3$	0.2	>17	17	
36	5	$CH{=}CH_2$[f]	H	116–117	33	$C_{20}H_{24}N_2O_3$	0.2	0.8	18	1.6

[a] Confidence limits $p = 75\%$. [b] The elemental analyses (C, H, N) for all new compounds were within ±0.4% of the theoretical values. [c] Rhinovirus type-2. [d] Mean MIC for five serotypes. [e] Maximum testable level (highest concentration of compound that causes no apparent effects on the cell monolayers). [f] Prepared from **29** (see Experimental Section). [g] Prepared from **20**. [h] Inactive.

Table III. Expanded Spectra against 15 Rhinovirus Serotypes

compd no.	in vitro activity: MIC, μM 1A	1B	2	6	14	15	21	22	25	30	41	50	67	86	89	MIC_{80},[a] μM
10	7.0	IA[b]	1.1	0.06	0.7	1.5	1.2	0.9	IA[b]	0.3	15.0	1.5	2.4	0.2	0.4	7.0
12	1.0	0.2	0.06	0.6	2.4	0.2	0.07	0.1	2.4	0.07	3.5	0.2	0.23	0.9	0.03	1.0
15	2.0	0.3	0.09	0.6	0.6	0.2	0.06	0.2	6.7	0.05	4.3	0.3	0.2	0.6	0.06	0.6
19	0.5	0.1	0.05	1.1	2.0	0.2	0.01	0.05	1.8	0.08	1.4	0.1	0.2	1.4	0.02	1.4
21	0.4	0.04	0.03	1.8	3.0	0.2	0.03	0.07	0.2	0.3	0.2	1.1	0.2	0.03	0.2	1.1
22	2.0	0.09	0.06	3.2	1.1	0.4	0.04	0.1	4.0	0.1	6.7	0.1	0.3	1.9	0.07	2.0
25	0.9	0.2	0.06	1.6	1.8	0.3	0.06	0.1	1.9	0.1	2.0	0.2	0.2	2.0	0.06	1.8
26	6.7	1.8	0.3	1.0	1.7	0.6	0.2	0.8	IA[b]	0.2	14.0	0.4	1.0	1.9	0.7	1.9
27	1.4	0.1	0.08	1.7	3.4	1.6	0.4	0.5	3.4	0.8	3.4	0.4	1.1	2.1	0.6	2.1
28	2.2	0.3	0.1	5.1	7.3	0.4	5.3	0.1	2.2	0.3	2.9	0.2	0.6	6.5	2.0	5.1
31	0.8	0.08	0.05	0.3	2.0	0.2	0.05	0.05	0.9	0.09	2.0	2.0	0.2	1.7	0.02	2.0
36	1.2	0.3	0.2	2.4	1.7	0.7	0.09	0.5	1.7	0.1	1.3	1.2	0.4	1.6	0.1	1.6

[a] The concentration that inhibits 80% of the serotypes tested. [b] Inactive.

substituents. For our initial examination of this data, a subset of compounds was selected that consisted of compounds having a measured MIC against each of the five serotypes tested. Because these compounds represent a relatively close series of analogues, molecular weight (MW) was chosen as a bulk descriptor. Baker[16] has suggested that molecular weight might be a useful measure of bulk, particularly for those compounds that interact with receptors. We hypothesize that the mode of action of **1** and its analogues involves interaction with a specific binding site on the virion capsid and that bulk is a physicochemical parameter warranting consideration. The lipophilic nature of the molecules was represented in the traditional manner by the partition coefficients (log *P*). The decision to use the Hammett sigma (σ) as an indicator of the electronic nature of the substituent was complicated by the fact that these substituents are ortho to the alkyl ether. Others have

Table IV. Regression Analysis

no.	equation[a]	*n*	*r*	*s*
1	$\log [1/\overline{MIC}] = 5.63(\pm 1.15)MW - 5.15 (\pm 1.03)$	13	0.81	0.24
2	$\log [1/\overline{MIC}] = 4.97 (\pm 1.04)MW + 1.06 (\pm 0.48) \log P - 5.44 (\pm 0.88)$	13	0.88	0.21
3	$\log [1/\overline{MIC}] = 2.46 (\pm 1.11)MW + 1.47 (\pm 0.37) \log P + 0.50 (\pm 0.16)\sigma_m - 3.65 (\pm 0.88)$	13	0.94	0.16

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

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Table V. Physicochemical Constants and Antirhinovirus Activity

compd no.	substituent	log P^a	$\sigma_m{}^b$	MW[c]	mean MIC, μM	
					obsd[d]	calcd[e]
10	H	3.83		0.799	5.2	3.44
12	Cl	4.30	0.37	0.887	1.0	0.88
15	CH_3	4.40	−0.07	0.835	1.4	2.22
19	Br	4.42	0.39	1.000	0.4	0.42
20	NH_2	2.83	−0.16	0.837	7.0	8.40
21	CF_3	4.38	0.43	0.972	0.4	0.43
22	C_2H_5	4.71	−0.07	0.870	1.8	1.44
25	NO_2	3.49	0.71	0.914	0.7	0.72
26	CH_3O	3.44	−0.12	0.876	4.5	2.74
27	$CH_2CH{=}CH_2$	4.93	−0.13[f]	0.901	1.1	1.11
28	CH_3CO	3.28	0.38	0.906	1.1	1.45
31	CHF_2	4.59	0.29	0.926	1.0	0.63
36	$CH{=}CH_2$	4.65	0.05	0.865	0.8	1.27

[a] Determined via RPHPLC-retention-time procedure,[23] $n = 3$, mobile phase pH 7.4. Those compounds not measured were estimated by use of π[18] with log P of close structural analogues. [b] See ref 18. [c] Normalized to compound **19**. [d] See Table II. [e] Obtained from eq 3. [f] Calculated from F_M and R_M.

Table VI. Parameter Correlation Matrix

	log P	σ_m	MW
log P	1.000		
σ_m	−0.051	1.000	
MW	0.287	0.670	1.000

raised questions regarding the applicability of the Hammett equation to ortho-substituted compounds.[17] Since all the compounds in our data set had the same substitution pattern, any error introduced would be internally consistent and therefore canceling. In this example, either sigma meta (σ_m) or sigma ortho (σ_o) could be considered. However, only the results for σ_m[18] are included in Table V since the alternative σ_o equation would give approximately the same results due to the high correlation (r = 0.98) between these two parameters for our data set.

There was a good correlation (r = 0.81) between $\overline{\text{MIC}}$ and MW as shown in eq 1, Table IV, indicating a strong dependence on bulk for activity. The equation was further improved (r = 0.88) by including the log P term: eq 2, Table IV. The three-parameter equation 3 gave a higher correlation (r = 0.94) upon the addition of the σ_m term and suggested the relative importance of each term to the overall observed $\overline{\text{MIC}}$. The low interdependence of these three parameters is shown in Table VI.

The values calculated for $\overline{\text{MIC}}$ by using eq 3 are shown in Table V. In general, these values compared well to the observed $\overline{\text{MIC}}$.

Discussion

The five serotypes included in the $\overline{\text{MIC}}$ represent the previously reported range of antiviral sensitivities to compounds of this class.[1,19] The $\overline{\text{MIC}}$ correlates well (r = 0.83) with the MIC_{80}. Furthermore, when compound **12**, for example, was tested against 33 serotypes, the MIC_{80} was determined to be 0.9 μM, which was very close to the 1.0 μM value determined for the 15 serotypes. These results suggest that the original five serotypes that we had chosen for the secondary evaluation are predictive of the spectrum of activity of these compounds.

The structure–activity relationships that have emerged from this series of compounds indicate that antirhinovirus activity is dependent upon several factors such as bulk, lipophilicity, and the location of and the electronic effect due to substituents on the phenyl ring. These parameters have been studied by a regression analysis which gives a good correlation with $\overline{\text{MIC}}$.

The effect on activity of moving the chloro or methyl group from the 2- to the 3-position (compounds **12**, **16**, **15**, and **17**) was unexpected. This effect was particularly dramatic with respect to the $\overline{\text{MIC}}$. The mode of action of these compounds suggests that they bind to a site on the viral capsid. It is conceivable that binding of the drug to the capsid is strongly dependent upon the substitution pattern on the phenyl ring, as well as the other parameters encompassed within our regression analysis.

Our investigation in this area has resulted in the synthesis of some very potent compounds. As more information is generated concerning the nature and function of the binding site on the viral capsid, the greater the chance will be of successfully designing more broad-spectrum antirhinovirus agents.

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 JEOL FX-270 spectrophotometer and the mass spectra on a JEOLCO double-focusing high-resolution mass spectrophotometer.

General Methods of Synthesis. 3-Chloro-4-hydroxy-*N*-(2-hydroxyethyl)benzamide (4: **X = Cl, Y = H**). A mixture of 23.2 g (0.12 mol) of ethyl 3-chloro-4-hydroxybenzoate and 14.8 g (0.24 mol) of 2-aminoethanol was heated with stirring to 135 °C for 2 h. After cooling, the viscous oil was treated dropwise at 15 °C with 125 mL of 6 N HCl. After stirring, a solid precipitate was collected by filtration, washed with cold water, and dried to give 17.8 g (82%) of the hydroxyethyl amide 4, mp 118–122 °C.

3-Chloro-4-(4,5-dihydro-2-oxazolyl)phenol Hydrochloride (**5a**). To a suspension of 20.0 g (0.092 mol) of 4 in 200 mL of *i*-PrOAc was added dropwise with stirring at 25 °C 17.8 g (0.15 mol) of thionyl chloride. After the mixture was stirred for 1.5 h, the resulting solid was collected, washed with *i*-PrOAc, and dried to give 21 g (97.7%) of **5a**: mp 153–155 °C; NMR (Me_2SO-d_6) δ 3.6–4.4 (4 H, m, OCH_2, NCH_2), 6.61 (1 H, dd, J = 12 Hz, 2 Hz, aromatic), 6.71 (1 H, d, J = 2 Hz, aromatic), 7.45 (1 H, d, J = 12 Hz, aromatic). Anal. ($C_9H_8ClNO_2$·HCl) C, H, N.

5-[5-[2-Chloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]-pentyl]-3-methylisoxazole (**12**). A mixture of 7.5 g (0.032 mol) of **5a**, 7.4 g (0.032 mol) of 5-(5-bromopentyl)-3-methylisoxazole,[19] 4.8 g (0.032 mol) of NaI, and 9.7 g (0.07 mol) of anhydrous K_2CO_3 in 200 mL of CH_3CN was heated to reflux with stirring for 24 h. The suspended solids were removed by filtration and washed with CH_3CN. Removal of the solvent in vacuo gave a semisolid, which was partitioned between CH_2Cl_2 and H_2O. The organic layer was

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dried, the solvent removed, and the residual solid recrystallized from *i*-PrOH to give 7.4 g (65.8%) of **12**: mp 102–104 °C; NMR ($CDCl_3$) δ 1.4–2.0 (6 H, m, 3 CH_2), 2.26 (3 H, s, CH_3), 2.76 (2 H, t, J = 7 Hz, CH_2), 3.9–4.6 (6 H, m, 2 OCH_2, NCH_2), 5.83 (3 H, s, CH), 6.89 (1 H, d, J = 9 Hz, aromatic), 7.79 (1 H, dd, J = 9 Hz, aromatic), 7.95 (1 H, d, J = 2 Hz, aromatic). Anal. ($C_{18}H_{22}N_2O_3$) C, H, N.

5-(4,5-Dihydro-2-oxazolyl)-2-[[5-(3-methyl-5-isoxazolyl)pentyl]oxy]benzenemethanol (30). To a solution of 2.3 g (3.5 mmol) of **29** in 10 mL of CH_3OH was added portionwise with stirring at room temperature 200 mg (5.3 mmol) of $NaBH_4$. After the addition was complete, the solution was stirred at room temperature for 1 h and then treated with acetic acid dropwise until the solution was slightly acidic. After the solution was diluted with 50 mL of H_2O, the precipitated solid was collected and recrystallized from EtOAc to give 1.2 g (99%) of **30**: mp 140–141 °C; NMR ($CDCl_3$) δ 4.7 (2 H, s, CH_2OH). Anal. ($C_{19}H_{24}N_2O_4$) C, H, N.

5-[5-[2-(Difluoromethyl)-4-(4,5-dihydro-2-oxazolyl)phenoxy]pentyl]-3-methylisoxazole (31). To a solution of 3.2 g (0.01 mol) of (diethylamino)sulfur trifluoride (DAST) in 10 mL of CH_2Cl_2 was added dropwise 3.0 g (0.01 mol) of **29** in 5 mL of CH_2Cl_2 at room temperature. The solution was stirred for 24 h, diluted with an additional 50 mL of CH_2Cl_2, extracted with H_2O, and dried. Removal of the solvent gave a residue, which was purified by MPLC[21] (EtOAc). The solid obtained was recrystallized from *i*-PrOAc to give 1 g of **31** (27%): mp 88–89 °C; NMR ($CDCl_3$) δ 6.6 (1 H, s, CHF_2). Anal. ($C_{19}H_{22}F_2N_2O_3$) C, H, N.

5-[5-[2-Ethenyl-4-(4,5-dihydro-2-oxazolyl)phenoxy]pentyl]-3-methylisoxazole (36). To a suspension of 5.2 g (0.015 mol) of triphenylmethylphosphonium bromide in 75 mL of THF was added at 0 °C under nitrogen 10 mL of 1.55 M *n*-butyllithium.[22] After the addition was complete, the mixture was stirred at room temperature for 2 h, and then 5.0 g (0.0146 mol) of **29** in 25 mL of THF was added dropwise. The resulting suspension was left at room temperature for 18 h. The suspended solid was removed by filtration and the filtrate concentrated to dryness. The residual solid was purified by MPLC ($CHCl_3$) in order to remove triphenylphosphine oxide. Recrystallization of the resulting solid from EtOAc gave 1.8 g of **36** (33%): mp 116–117 °C; NMR ($CDCl_3$) δ 5.25–5.35, 5.80–5.90 (2 H, d, C=CH_2), 6.95–7.15 (1 H, d, CH=C cis and trans). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

5-(4,5-Dihydro-2-oxazolyl)-2-[[5-(3-methyl-2-isoxazolyl)pentyl]oxy]benzoic Acid (32). To a solution of 4.0 g (12 mmol) of **29** in 60 mL of ethanol was added at room temperature 6 mL of a 4.6 M solution of aqueous silver nitrate, and the mixture was stirred for 15 min.[23] Sixty milliliters of a 1.0 M solution of aqueous KOH was added slowly, and the mixture was stirred for 2 h. The mixture was filtered and acidified with concentrated HCl to pH 2. The precipitate was filtered and the filter cake washed with 1 M HCl. The resulting paste was dried in vacuo briefly and recrystallized from CH_3OH to yield 1.6 g (36%) of **32**: mp 170–171 °C; NMR (Me_2SO-d_6) δ 12.8 (1 H, s, COOH). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

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Registry No. 1, 87495-31-6; 4 (X = Cl, Y = H), 98033-80-8; **5** (X = Y = H), 81428-58-2; **5** (X = CF_3, Y = H), 105639-29-0; **5** (X = $CH_2CH=CH_2$, Y = H), 105639-30-3; **5a**, 98033-81-9; **5b**, 98033-92-2; **5c**, 105639-19-8; **5d**, 105639-20-1; **5e**, 98034-05-0; **5f**, 105639-21-2; **5g**, 105639-22-3; **5h**, 105639-23-4; **5i**, 105639-24-5; **5j**, 98033-64-8; **5k**, 105639-25-6; **5l**, 105639-26-7; **5m**, 105639-27-8; **5n**, 98033-59-1; **5o**, 105639-28-9; **10**, 98034-30-1; 11, 98033-98-8; **12**, 98033-68-2; **13**, 98033-87-5; 14, 98033-82-0; **15**, 105639-02-9; **16**, 98033-68-2; **17**, 105639-03-0; **18**, 98033-69-3; **19**, 105639-04-1; **20**, 105639-05-2; **21**, 105639-06-3; **22**, 105639-07-4; **23**, 105639-08-5; **24**, 105639-09-6; **25**, 105639-10-9; **26**, 98033-66-0; **27**, 105639-11-0; **28**, 105639-12-1; **29**, 105639-13-2; **30**, 105639-14-3; **31**, 105639-15-4; **32**, 105639-16-5; **34**, 105639-17-6; **35**, 105639-18-7; **36**, 105663-64-7; $H_2N(CH_2)_2OH$, 141-43-5; 5-(4-bromobutyl)-3-methylisoxazole, 98033-94-4; 5-(5-bromopentyl)-3-methylisoxazole, 98033-85-3; 5-(6-bromohexyl)-3-methylisoxazole, 98033-83-1; 5-(7-bromoheptyl)-3-methylisoxazole, 91945-38-9; ethyl 3-chloro-4-hydroxybenzoate, 16357-41-8.

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Octahydropyrazino[2′,3′:3,4]pyrido[1,2-*a*]indoles. A New Class of Potent Antihypertensive Agents

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Simplifications and modifications of the vincamine molecule led to the discovery of antihypertensive 1,2,3,4,4a,5,6,12b-octahydro-12-methylpyrazino[2′,3′:3,4]pyrido[1,2-*a*]indoles. Stereoselective syntheses of both 4a,12b-cis and 4a,12b-trans isomers represent new annulation strategies for the construction of fused piperazines. Compounds of the trans series were at least 10 times more potent than the corresponding cis isomers. Antihypertensive activity and α_1-adrenoceptor blocking properties peaked with a simultaneous introduction of 4-methylethyl and 1-alkyl substituents. Compound **15j** (AY-28,228; atiprosin), (4a,12b-*trans*)-1-ethyl-1,2,3,4,4a,5,6,12b-octahydro-12-methyl-4-(1-methylethyl)pyrazino[2′,3′:3,4]pyrido[1,2-*a*]indole, was chosen for a detailed preclinical evaluation.

Over the years, the family of indole alkaloids has been a source of naturally occurring antihypertensive principles, and structural prototypes possessing the indole nucleus have given rise to a number of useful drugs. Among the more recently studied indole alkaloids are those of the *Vinca* species. Vincamine (**1**) has interesting cardiovascular properties,[1-4] exhibits moderate antihypertensive effects[1,2] through its vasodilative mode of action,[2,3] and appears to be successful in the treatment of pathological

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