nm (ε 32 000), 265 (ε 21 000); NMR (Me_2SO-d_6) δ 1.4 (s, 9 H), 2.01 and 2.05 (2 s, 3 H), 3.48 (m, 2 H), 5.10 (2 d, 1 H), 5.38 (s, 2 H), 5.70 (m, 2 H), 7.3–8.3 (m, 9 H), 9.18 (m, 2 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N.

Separation of *R* and *S* Isomers of 7 by Chromatography. Four grams of *p*-nitrobenzyl (*RS*)-7-[*N*-(*tert*-3-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (7) was dissolved in 500 mL of dichloromethane and slurried with 20 g of silica gel 60. The solvent was removed and the mixture was added to an 8 cm × 15 cm column packed with 400 g of silica gel 60 in toluene. The column was eluted with a gradient of 2 L of 5% ethyl acetate in toluene (v/v) to 2 L of 15% ethyl acetate in toluene and finally with 3 L of 15% ethyl acetate in toluene. Twenty-five-milliliter fractions were collected every 2 min. NMR and thin-layer chromatographic analysis showed partial separation of the *R* and *S* isomers, resulting in 1.44 g of *p*-nitrobenzyl (*R*)-7-[*N*-(*tert*-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (72% yield), 1.135 g of the *RS* mixture, and 1.23 g of the *S* isomer, all as colorless solids. *R* isomer: IR ($CHCl_3$) 1785 cm^{-1} (β-lactam carbonyl); UV λ_{max} (MeOH) 264 nm (ε 21 000); MS, *m/e* 538, 539 (M + 1, + 2); NMR (Me_2SO-d_6) δ 1.39 (s, 9 H), 2.01 (s, 3 H), 3.2–3.7 (AB q, *J* = 18.4 Hz, 2 H), 5.07 (d, *J* = 4.8, Hz 1 H), 5.38 (s, 2 H), 5.65–5.85 (m, 2 H), 7.2–8.3 (m, 10 H), 9.21 (d, *J* = 7.5 Hz, 1 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N. *S* isomer: IR ($CHCl_3$) 1783 cm^{-1} (β-lactam carbonyl); UV λ_{max} (MeOH) 262 nm (ε 20 100); MS, *m/e* 539 (M + 1); NMR (Me_2SO-d_6) δ 1.39 (s, 9 H), 2.05 (s, 3 H), 3.3–3.8 (AB q, 2 H), 5.13 (d, *J* = 4.8, Hz, 1 H), 5.36 (s, 2 H), 5.6–5.8 (m, 2 H), 7.15–8.3 (m, 10 H), 9.12 (d, *J* = 7.9 Hz, 1 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N.

(*S*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylic Acid (1S). Following the procedure described for the *R* epimer,[1] the Boc and *p*-NB groups were removed from the diprotected *S* epimer of 7, yielding a colorless solid: NMR (Me_2SO-d_6) δ 2.0 (s, 3 H, 3-CH_3), 3.45 (AB q, 2 H, C_2 H_2), 4.95 (s, 1 H, α-H), 5.04 (d, 1 H, C_6 H), 5.57 (d, 1 H, C_7 H), 7.25–8.06 (m, 5 H, arom). Anal. ($C_{18}H_{17}N_3O_4S_2$) C, H, N.

***p*-Nitrobenzyl (*RS*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate (8).** A solution of 9.6 g (15 mmol) of *p*-nitrobenzyl 7-[*N*-(*tert*-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate in 210 mL of acetonitrile containing 3.42 g (18 mmol) of *p*-toluenesulfonic acid monohydrate was stored at 25 °C for 3 days. The precipitate that formed was collected by filtration and identified as *p*-nitrobenzyl (*RS*)-7-(3-benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate *p*-toluenesulfonic acid salt monohydrate: IR (KBr) 1777cm^{-1}; UV λ_{max} (EtOH) 263 nm (ε 18 500); MS, *m/e* 539; NMR (Me_2SO-d_6) 2.0 (s, 3 H), 2.3 (s, 3 H), 3.15–3.65 (AB q, *J* = 18, Hz, 2 H), 3.32 (s, 2 H), 5.0 d, 1 H), 5.35 (s, 2 H), 5.5 (s, 1 H), 5.8 (dd, 1 H), 7.0–8.3 (m, 13 H), 9.55 (d, 1 H). Anal. ($C_{32}H_{30}N_4O_9S_3 \cdot H_2O$) C, H, N, O, S.

The salt was dissolved in 60 mL of 10% aqueous sodium bicarbonate and the solution was extracted several times with ethyl acetate. The extracts were combined, washed with water, dried, and concentrated to dryness by evaporation under reduced pressure to give 7.9 g (76% yield) of *p*-nitrobenzyl (*RS*)-7-(3-benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate as a colorless gum: NMR (Me_2SO-d_6) β 1.99 and 2.04 (2 s, 3 H, *R* and *S* isomers), 3.26–3.6 (m, 2 H), 4.9 (s, 1 H), 5.1 (d, 1 H), 5.36 (s, 2 H), 5.7 (m, 1 H), 7.3–8.25 (m, 10 H).

(*RS*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylic Acid (1). A solution of 5.2 g of *p*-nitrobenzyl 7-(3-benzothienylglycylamido)-3-cephem-4-carboxylate in 150 mL of CH_3OH containing 10 mL of 1 N HCl and 5.2 g of 5% palladium on carbon was stirred at 25 °C for 90 min under 60 psi H_2. The reaction mixture was filtered and the filtrate was concentrated to give a gum. The gum was dissolved in 40 mL of water and 40 mL of EtOAc. The mixture was neutralized to pH 7.0 by addition of 1 N NaOH, and the organic layer was removed and discarded. The aqueous layer was acidified to pH 4.25 by addition of 1 N HCl. The aqueous acid solution was lyophilized to afford 1.62 g of (*RS*)-7-(3-benzothienylglycylamido-3-methyl-3-cephem-4-carboxylic acid. Separation of the isomers was effected by high-performance liquid chromatography to give 299.2 mg of *R*, 131.7 mg of *S*, and 106.6 mg of *RS* isomers as colorless amorphous solids. NMR (Me_2SO-d_6) of *R* isomer: δ 1.94 (s, 3 H), 3.20 and 3.43 (AB q, *J* = 19.5 Hz, 2 H), 4.96 (d, *J* = 4.84 Hz, 1 H), 5.07 (s, 1 H), 5.62 (dd, *J* = 4.4 Hz, 1 H), 7.2–8.1 (m, 6 H). Anal. ($C_{18}H_{17}N_3O_4S_2$) C, H, N.

Acknowledgment. We express our appreciation to Dr. Lowell D. Hatfield for valuable discussions and guidance, to Donnis M. Berry and Lyell Huckstep for analytical and preparative HPLC, and to the analytical and chemistry sections for elemental analyses, UV, IR, and NMR spectroscopy.

[[(4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles. Inhibitors of Picornavirus Uncoating†

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Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received March 18, 1985*

A series of [[(4,5-dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles has been synthesized and evaluated as antipicornavirus agents. The effect of alkyl groups in the 4- and 5-position of the oxazoline ring, as well as the alkyl chain length, on antiviral activity was examined. Compound **14** was evaluated in vivo and was found to significantly reduce mortality at an oral dose of 4 mg/kg in mice infected intracerebrally with poliovirus-2. Compound **14** was also effective in preventing paralysis when administered intraperitoneally to mice infected subcutaneously with a lethal dose of ECHO-9 virus. On the basis of the results of these studies, compound **14** is a strong candidate for clinical evaluation as a systemic agent for the treatment of picornavirus infections.

We recently reported the synthesis and antipicornavirus activity of a new class of compounds whose mode of action was determined to involve the prevention of viral uncoating.[1] In continuation of our pursuit of compounds with this mode of action, we have prepared several modifications of our original series with increased potency both in vitro and in vivo.

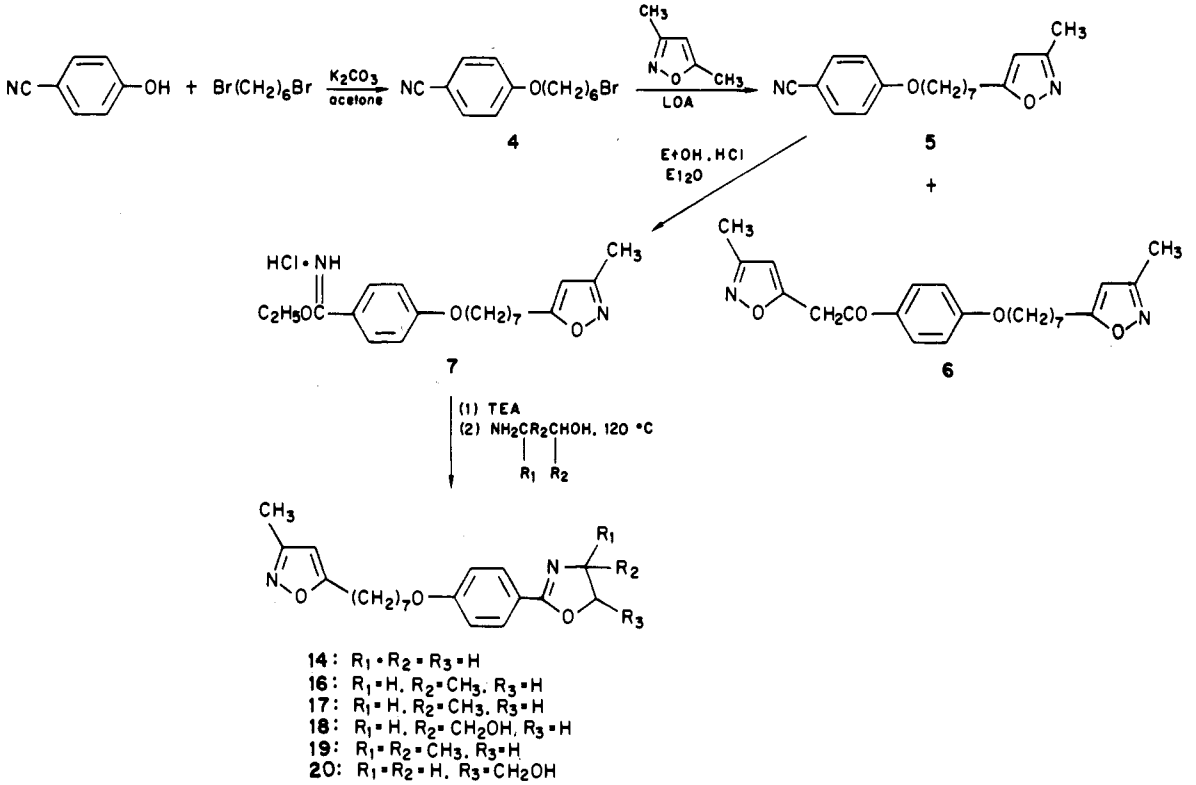
As a result of our previous work, the isoxazole **1** was shown to have broad spectrum activity against several serotypes of rhino and enteroviruses with MICs as low as

† Presented in part at the VIIIth International Symposium on Medicinal Chemistry, Upsalla, Sweden, Aug 1984.

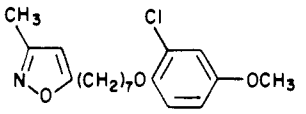
(1) Diana, G. D.; McKinlay, M. A.; Brisson, C. J.; Zalay, E. S.; Miralles, J. V.; Salvador, U. J. *J. Med.* **1985**, *28*, 748.

0022-2623/85/1828-1906$01.50/0

Scheme I

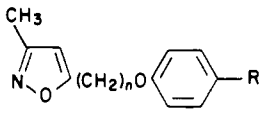


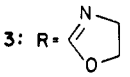
0.1 μg/mL against the former and 0.04 μg/mL against the latter.[1] When administered orally to mice infected in-



tracerebrally with poliovirus type-2 (polio-2), compound 1 exhibited a minimal protective dose (MPD) of 31 mg/kg.[2] Although compound 1 demonstrated potent activity in vitro, a high MPD was observed in mice presumably due to low plasma levels. Our objective was to synthesize an orally effective agent with an MPD against polio-2 in mice significantly less than that of compound 1.

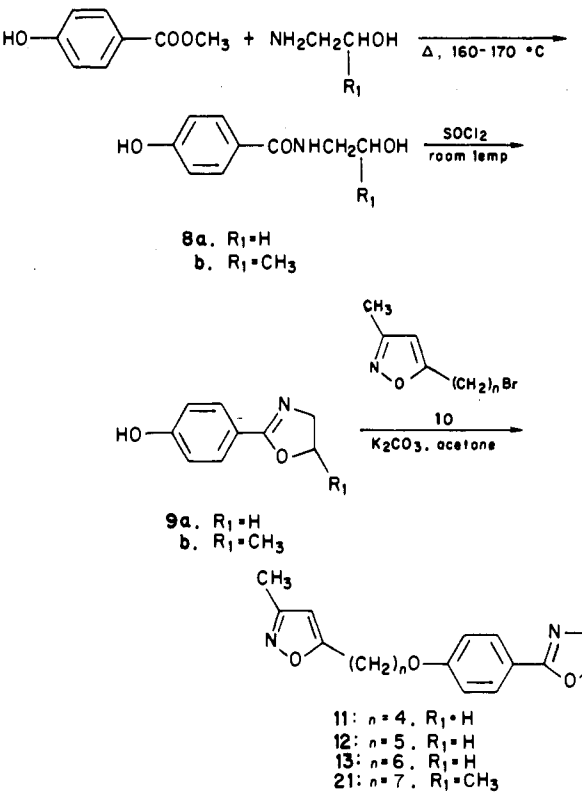
We previously found that the carbethoxy homologue **2**, when tested in vitro, exhibited greater potency than 1 against rhino-2 and was also active against polio-2 virus.[1]





No efficacy, however, was found when **2** was administered orally to mice at doses up to 100 mg/kg. The possibility exists that **2** was hydrolyzed to the corresponding acid, which is inactive in vitro. Several modifications of the ester group were made without any improvement in systemic activity. The oxazoline **3** was prepared as a cyclic variation of the ethyl ester having similar space-filling requirements, which necessitated replacing one of the oxygens with a nitrogen atom.

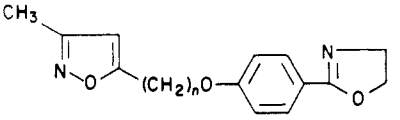
Scheme II



Chemistry. Initially, the oxazolines were synthesized according to the method used in Scheme I. The requisite (4-cyanophenoxy)alkyl bromides were prepared according to published procedures.[3] Lithiation of 3,5-dimethyl-

(2) McKinlay, M. A.; Brisson, C. J.; Miralles, J. V.; Diana, G. D. Paper presented at the 23rd Intersicence Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, NV, Oct 1983.

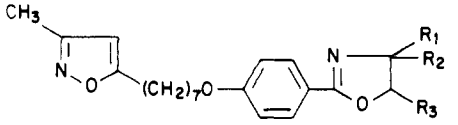
Table I. Effect of Chain Length on Antipicornavirus Activity



compd	n	mp, °C	method	yield, %	formula[b]	in vitro act.:[a] MIC μg/mL RV-2[c]	polio-2[d]	MTL[e]
11	4	93–94	B	75	$C_{17}H_{20}N_2O_3$	0.53	0.490	6.2
12	5	95–96	B	69	$C_{18}H_{22}N_2O_3$	0.1	0.003	3.1
13	6	88–89	B	76	$C_{19}H_{24}N_2O_3$	0.12	0.008	3.1
14	7	89–90	A	79	$C_{20}H_{26}N_2O_3$	0.10	0.004	6.2
15	8	73–74	B	55	$C_{21}H_{28}N_2O_3$	0.16	0.010	8

[a] Confidence limits $p = 75\%$. [b] The elemental analyses (C, H, and N) for all new compounds were within ±0.4% of the theoretical values. [c] Rhinovirus type-2. [d] Poliovirus type-2. [e] Maximum testable level.

Table II. Effect of Substitution of Oxazoline Ring on Antipicornavirus Activity



compd	R_1	R_2	R_3	mp, °C	method	yield, %	formula[a]	in vitro act.: MIC, μg/mL RV-2[b]	polio-2[c]	MTL[d]
14	H	H	H					0.1	0.004	6.2
16	H	CH_3	H	72	A	71	$C_{21}H_{28}N_2O_3$	0.04	0.12	3.1
17	H	CH_3(D)	H	71	A	69	$C_{21}H_{28}N_2O_3$	0.02	0.16	3.1
18	H	CH_2OH	H	77–78	A	50	$C_{21}H_{28}N_2O_4$	0.90	IA[f]	3.1
19	CH_3	CH_3	H	41–42	A	74	$C_{22}H_{30}N_2O_3$	0.06	1.1	3.1
20	H	H	CH_2OH	75–76	A	61	$C_{21}H_{28}N_2O_4$	1.6	2.6	3.1
21	H	H	CH_3	81–82	B	50	$C_{21}H_{28}N_2O_3$	0.06	0.49	6.2
22	H	H	CH_2OCH_3	60–61	e	80	$C_{22}H_{30}N_2O_4$	0.20	IA	3.1

[a] The elemental analyses (C, H, and N) for all new compounds were within ±0.4% of the theoretical values. [b] Rhinovirus type-2. [c] Poliovirus type-2. [d] Maximum testable level. [e] Prepared from compound 20. [f] Inactive.

isoxazole at −70 °C followed by treatment with 4 gave 5 in 23% yield, in addition to 3% of 6. The nitrile 5 was converted to the imino ester 7 under standard conditions in 97% yield. Compound 7 was converted to its free base and then heated with 1 equiv of various aminoethanols to give the corresponding oxazoline.

Subsequently, an alternative approach, which resulted in greater overall yields, was used and is shown in Scheme II. Methyl 4-hydroxybenzoate was heated with the appropriate amino alcohol to give amides 8 which on treatment with thionyl chloride in isopropyl acetate at room temperature[4] gave 9 in 96% yield. Alkylation of 9 with the (bromoalkyl)isoxazoles[5] 10 gave the oxazolines in approximately 70% yield.

Biology. In our previous series we had established that a six- or seven-carbon chain was required for optimum activity against both rhino-2 and polio-2. In order to determine the requirements for optimum activity in this series, the carbon chain length was varied from four to eight (Table I). Compounds were screened in the plaque-reduction assay which was previously described.[1] The five- and seven-carbon chain homologues exhibited the greatest activity against polio-2 while the six-carbon homologue exhibited slightly less activity. All three compounds, however, were equipotent against rhino-2. The four- and eight-carbon homologues were considerably less effective against both viruses.

Table III. Antipicornavirus Activity

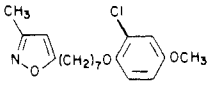
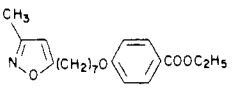
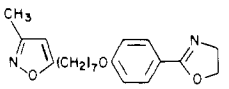
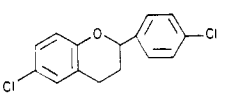
compd		MIC, μg/mL RV-2	polio-2
1		0.04	0.05
2		0.025	>0.8
14		0.10	0.004
BW 638C		0.09	IA

Table IV. Prevention of Poliovirus-Induced Death by Oral Medication with Compound 14[a]

dose, mg/kg	% survivors[b]	no. survivors/total
32	71 ± 5.0	15/20
16	65 ± 5.5	13/20
8	45 ± 11.5	9/20
4	40 ± 11.5	8/20
placebo	20 ± 8.5	4/20

[a] Treatment started 1 h prior to infection. [b] Test terminated after 20 days post-infection.

The effect on in vitro activity resulting from substitution on the oxazoline ring is shown in Table II. When compared to 14, none of the compounds was more effective against polio-2. However, increased activity was observed for the 4-methyl compounds 16 and 17 against rhino-2.

(3) Diana, G. D.; Salvador, U. J.; Zalay, E. S.; Johnson, R. E.; Collins, J. C.; Johnson, D.; Hinshaw, W. B.; Lorenz, R. R., Thielking, W. H.; Pancic, F. *J. Med. Chem.* **1977**, *20*, 750.
(4) Meyers, A. I.; Reuman, M.; Gabel, R. H. *J. Org. Chem.* **1981**, *46*, 783.
(5) Micetich, R. G. *Can. J. Chem.* **1970**, *48*, 2006.

Table V. Prevention of Echo-9-Induced Paralysis in Suckling Mice by Intraperitoneal Medication with 14[a]

dose, mg/kg	% nonparalyzed[b]	no. nonparalyzed/total
33	96.7 ± 5.2	29/30
10	63.7 ± 5.5	19/30
3.3	20 ± 1.0	6/30
1.0	6.7 ± 3.0	2/30
placebo	0	0/30

[a] Treatment started 1 h prior to infection. [b] Test terminated after 14 days post-infection.

The D isomer **17** exhibited slightly greater activity than the D,L mixture; however, there was no difference in activity against polio-2. The 4,4-dimethyl homologue **19** was also slightly more effective than **14** against rhino-2. Methylation in the 5-position of the oxazoline ring, **21**, again reduced activity against polio-2, as did hydroxymethylation in the 4- and 5-position, compounds **18** and **20**, respectively. The latter compounds were also less effective than **14** against rhino-2. The 5-methoxymethyl compound, **22**, was devoid of activity against polio-2.

4′,6-Dichloroflavan[6] has been shown to inhibit rhinovirus replication in vitro[7] and has undergone clinical trials.[8] Compounds **1**, **2**, and **14** and 4′,6-dichloroflavan were evaluated against rhino-2 and polio-2, and the results are shown in Table III. Although **14** exhibited less potency than **1** in vitro against rhino-2, its activity against polio-2 was 10-fold greater. Compound **2** was only weakly effective against polio-2 while 4′,6-dichloroflavan was inactive against this virus.

Since the original objective was to enhance in vivo activity, the efficacy of compound **14** was assessed in mice medicated orally, beginning 1 h prior to intracerebral infection with polio-2 according to the procedure previously described.[1] The results are shown in Table IV. At a dose of 4 mg/kg, 40% of the infected mice survived while at 8, 16, and 32 mg/kg, the relative survival rates were 45, 65, and 71%, respectively, as compared to a 20% survival rate for the placebo-treated animals. When therapy was delayed to 48 h post-infection at 100 mg/kg, t.i.d., 60% of the animals survived compared to the placebo-treated group in which there were no survivors.

Efficacy of compound **14** was assessed in an additional animal model in which suckling mice were infected subcutaneously with ECHO-9 virus according to the procedure described in the Experimental Section. Initially, the animals were medicated ip prior to infection, similar to the polio-2 experiment, and then once daily for 8 days. The results are shown in Table V. At 1.0 mg/kg, 6.7% of the animals were protected from paralysis. At doses of 3.3, 10, and 33 mg/kg, the percentages of nonparalyzed animals were 20, 63.7, and 96.7, respectively. All of the placebo-treated animals were paralyzed by the end of the test.

With delayed therapy, 100 mg/kg prevented paralysis in 75% of the animals and 33 mg/kg protected 25%, while all of the placebo-treated animals were paralyzed.

Discussion

It is interesting to note the effect of substitution on the oxazoline ring on the activity against both screening viruses. When compared to **14**, the addition of lipophilic (methyl) groups on the oxazoline ring substantially reduced the level of activity against polio-2 but increased the efficacy against rhino-2. However, the introduction of hydrophylic (hydroxymethyl) groups reduced activity against both viruses. Consequently, within the limitation of our study, substitution on the oxazoline ring generally leads to a reduction in activity against polio-2 with a corresponding increase in MIC against rhino-2. The variation in chain length, as we have observed in related series,[1,3] has a pronounced effect on antipicornavirus activity, and in this case the effect on the MIC is similar for both rhino-2 and polio-2.

Compound **14** exhibited a 10-fold increase in activity as compared to **1** when administered orally to mice infected intracerebrally with polio-2. This would strongly suggest that the drug is absorbed into the central nervous system. In addition, the compound was effective when administered ip to mice infected sc with ECHO virus.

Compound **14** has been shown to inhibit uncoating, an early event in the replication cycle of poliovirus,[9] by reversibly binding to the viral capsid proteins. The nature of the binding is currently under study. This compound is not virucidal and has no effect on virion adsorption.

Experimental Section

Melting points were run 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 Instranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Knoxville, TN. NMR spectra were determined on a Varian HA-100 spectrophotometer and the mass spectra on a JEOL double-focusing high-resolution mass spectrophotometer by S. Clemans.

Mouse Infection with ECHO- Virus. Mouse pups less than 24 h old were medicated intraperitoneally in groups of 12 using a 27-gauge needle with **14** in 1% gum tragacanth, 2 h prior to subcutaneous infection with 5 LD_{50} ECHO-9 (Barty) administered in the scapular region. Subsequent ip medications were given once daily for 8–10 days. Mouse pups were examined twice daily for evidence of paralysis and death and counted each time to determine whether the pups had been cannibalized. Efficacy in the drug-treated group was assessed relative to the placebo-medicated group.

To assess therapeutic efficacy, the tests were run in a similar fashion as in the prophylactic test, except that the initial medication was delayed until 40-h post-infection.

General Method of Synthesis. Method A. 4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzonitrile (5). To a solution of 8.73 g (0.09 mol) of 3,5-dimethylisoxazole in 250 mL of THF under nitrogen was added dropwise at −70 °C 56.4 mL (0.087 mol) of 1.55 M *n*-butyllithium in hexane. After the addition was complete, the mixture was stirred for $^1/_2$ h at −70 °C and then treated dropwise with 25.38 g (0.10 mol) of 4-[(6-bromohexyl)oxy]benzonitrile (**4**) in 50 mL of THF. During the addition the temperature was maintained at −60 °C. After the addition was complete, the mixture was stirred for 1 h at −50 °C, at 0 °C for 3 h, and finally at room temperature overnight. The mixture was concentrated to dryness, the residue partitioned between EtOAc and H_2O, and the organic layer washed with 20 mL of 2 N HCl and then dried. Removal of the solvent gave an oil that was heated at 110 °C (0.1 mm) to remove any residual volatile impurities, and the residue was treated with Et_2O. Solid formed which was filtered and recrystallized from ether–pentane to afford 6.2 g (23%) of **5**, mp 52–53 °C. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

Concentration of the mother liquors from the crystallization of **5** afforded an oil that, after column chromatography on silica gel (elution with EtOAc), produced 1.2 g of **6**, mp 110–112 °C. Anal. ($C_{23}H_{28}N_2O_4$) C, H, N. NMR ($CDCl_4$): δ 7.9 (d, 2 H, aromatic), 6.9 (d, 2 H, aromatic), 6.1 (s, 1 H, =CH), 5.8 (s, 1 H, =CH), 4.18 (s, 2 H, CH_2CO), 3.98–4.1 (t, 2 H, CH_2O), 2.6–2.8 (t,

(6) Burroughs Wellcome Designation, BW 683C.
(7) Bauer, D. J.; Selway, J. W. T.; Batchelor, J. F.; Tisdale, M.; Caldwell, I. C.; Young, D. A. B. *Nature* **1981**, *292*, 369.
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2 H, CH_2C=), 2.3 (s, 6 H, 2 CH_3C=), 1.7-2.1 (m, 6 H, 3 CH_2). Mass spectra: m/e 396 (M^+).

Ethyl 4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzimidate Hydrochloride (7). A solution of 12.7 g (0.043 mol) of **5** in 40 mL of EtOH and 80 mL of Et_2O was saturated at -70 °C with HCl gas, then allowed to warm to 0 °C, and left for 3 h. Finally the solution was left overnight at room temperature. Removal of the solvent in vacuo gave a solid residue that was triturated with ether and filtered to afford 15.6 g (95%) of **7**, mp 112-114 °C. Anal. ($C_{20}H_{28}N_2O_3$·HCl) C, H, N. NMR ($CDCl_3$): δ 5.8 (s, 1 H, =CH), 4.3 (m, 2 H, N=$COCH_2CH_3$).

5-[7-[4-(4,5-Dihydro-2-oxazolyl)phenoxy]heptyl]-3-methylisoxazole (14). To a solution of 2.40 g (0.0063 mol) of **7** in 250 mL of CH_2Cl_2 was added 7 g of TEA in 20 mL of CH_2Cl_2. After stirring for 1 h, the solution was washed with water and dried. The solvent was removed, leaving a solid. To the solid was added 0.432 g (0.007 mol) of ethanolamine, and the mixture heated in an oil bath with stirring at 120 °C at which point gas evolution (NH_3) was observed. After $1^1/_2$ h the melt was cooled and dissolved in hot *i*-ProAc (100 mL) and chilled. Solid formed and was collected: 1.7 g (79%) of **14** was obtained; mp 86-89 °C. Anal. ($C_{20}H_{26}N_2O_3$) C, H, N. NMR ($CDCl_3$): δ 5.79 (s, 1 H, =CH), 3.8-4.6 (m, 6 H, 2 OCH_2, NCH_2), 2.71 (t, 2 H, CH_2C=), 2.25 (s, 3 H, CH_3C=), 1.2-2.0 (m, 10 H, 5 CH_2).

Method B. 4-(4,5-Dihydro-2-oxazolyl)phenol (9a). To a slurry of 61.8 g (0.34 mol) of 4-hydroxy-*N*-(2-hydroxyethyl)-benzamide[10] in 500 mL of *i*-PrOAc was added dropwise 40 mL (0.54 mol) of $SOCl_2$. After stirring for 2 h, the mixture was filtered to give 65.2 g (96%) of **9a**, mp 160-162 °C. Anal. ($C_9H_9NO_2$·HCl) C, H, N.

5-(4-Bromobutyl)-3-methylisoxazole (10, *n* = 4). To a solution of 28 mL (0.2 mol) of diiscpropylamine in THF was added at -5 °C and under nitrogen 77 mL of 2.6 M *n*-butyllithium in hexane (0.2 mol). After the addition was complete, the solution was cooled to -60 °C and 19.6 mL of 3,5-dimethylisoxazole (0.2 mol) in 50 mL of THF was added dropwise. The mixture was stirred for an additional 1 h at -60 °C, then added, via a nitrogen purge, to 250 g (1.2 mol) of 1,3-dibromopropane in 100 mL of THF, and chilled to -60 °C with stirring. The mixture was allowed to gradually warm to room temperature and then stirred overnight. After quenching with 20 mL of saturated NH_4Cl solution, the mixture was extracted with 250 mL of *i*-ProAc and the extract washed with water and dried. Removal of the solvent and excess dibromopropane gave 33.2 g of brown oil, which was purified by HPLC using 1:1 EtOAc-cyclohexane to give 23.4 g (54%) of **10**. NMR ($CDCl_3$): δ 2.77 (t, 2 H, $BrCH_2$), 2.27 (s, 3 H, CH_3C=), 1.7-2.2 (m, 4 H, CH_2CH_2). Mass spectra: m/e 217 (M^+, 1 Br).

5-[4-[4-(4,5-Dihydro-2-oxazolyl)phenoxy]butyl]-3-methylisoxazole (11). A mixture of 6.0 g (0.037 mol) of **10** (*n* = 4) 8.1 g (0.041 mol) of **9a**, 25 g (0.18 mol) of milled K_2CO_3, and 5 g (0.031 mol) of NaI in 200 mL of CH_3CN was heated to reflux with stirring for 21 h. The mixture was filtered, the filtrate concentrated in vacuo, and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with 5% KOH solution and water and dried. Removal of the solvent gave a solid that was recrystallized from *i*-PrOAc to give 8.5 g of **11** (75%), mp 93-94 °C. Anal. ($C_{17}H_{20}N_2O_3$) C, H, N. NMR ($CDCl_3$): δ 7.9 (d, 2 H, aromatic), 6.9 (d, 2 H, aromatic), 5.8 (s, 1 H, =CH), 4.2-4.5 (m, 2 H, NCH_2), 3.8-4.2 (m, 4 H, 2 OCH_2), 2.8 (t, 2 H, CH_2C=), 2.3 (s, 3 H, CH_3C=), 1.7-2.1 (m, 4 H, CH_2CH_2).

5-[7-[4-[4,5-Dihydro-5-(methoxymethyl)-2-oxazolyl]phenoxy]heptyl]-3-methylisoxazole (21). To a suspension of 1.08 g (0.027 mol) of a 60% NaH dispersion in 50 mL of dry THF was added dropwise at 30 °C 6.6 g (.0177 mol) of **20** in 50 mL of THF at 30 °C. The resulting mixture was heated at gentle reflux for 15 min and then cooled to room temperature, and 4.3 g (0.03 mol) of CH_3I in 25 mL of THF was added dropwise during a 10-min period. The mixture was stirred at room temperature overnight. The solvent was removed, leaving a solid that was washed with pentane. The residual material was dissolved in EtOAc, washed with H_2O, and dried. After removal of the EtOAc an oil remained that solidified on standing. Recrystallization from hexane gave 5.3 g of **21** (80%), mp 60-61 °C. Anal. ($C_{22}H_{30}N_2O_4$) C, H, N. NMR ($CDCl_3$): δ 3.49 (s, 3 H, OCH_3), 2.77 (t, 2 H, CH_2C=), 2.31 (s, 3 H, CH_3C=), 1.3-2.1 (m, 10 H, 5 CH_2).

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Quantitative Structure-Activity Relationship of Antifolate Inhibition of Bacteria Cell Cultures Resistant and Sensitive to Methotrexate

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Sets of 5-(substituted benzyl)-2,4-diaminopyrimidines and 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3-substituted phenyl)-*s*-triazines as well as several other antifolates were tested as inhibitors of *Escherichia coli* dihydrofolate reductase and *E. coli* cell cultures both sensitive and resistant to methotrexate. From the results quantitative structure-activity relationships (QSAR) were formulated. The triazines were found to inhibit sensitive and resistant cell cultures to the same degree, but the benzylpyrimidines showed marked differences against the two types of cells. Increased hydrophobicity produced benzylpyrimidines more active against the resistant *E. coli* cell. Metroprine did not discriminate between the two types of cells cultures, but pyrimethamine and 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine (BW 301U) did. The results are compared with triazines and benzylpyrimidines acting on *Lactobacillus casei* and murine tumor cells sensitive and resistant to methotrexate. QSAR is shown to be an effective means for detecting receptor differences.

A most serious problem in drug research is the development of the means for making drugs effective against pathogenic cells which have become drug resistant. While there are a variety of ways one might approach such problems, we have been trying to understand the differences in the structure-activity relationships of antifolates acting on sensitive and resistant cells of bacterial and tumor origin.[1,2] Apart from metabolism there are several

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