washed three times in 95% EtOH, dried, and analyzed in a scintillation counter. The percent TK activity of control was determined by dividing the corrected counts per minute obtained in the presence of drug by those obtained in the absence of the drug (× 100).

Tumor Growth Inhibition Studies (by Dr. J. H. Burchenal). The technique of Fisher[45] was employed with modifications.[8] Mouse cell lines L1210/0, P815/0, and P815/Ara-C were incubated in McCoy's medium 5A with 15% fetal calf serum. The initial inoculum was 40000–60000 leukemia cells/mL. For growth inhibition studies, 0.1 mL of a 20-fold concentration of the nucleoside in question was added to 2 mL of media containing 4×10^4 cells/mL in Linbro tissue culture multiwell plates and allowed to incubate at 37 °C in 5% CO_2 for 96 h. Growth to approximately 10^6 cells/mL occurred in the control wells. The contents of each well were counted on a Coulter Counter and the percentage of inhibition of growth and the concentrations inhibiting cell growth by 50% were calculated.

(45) Fisher, G. A. *Ann. N.Y. Acad. Sci.* **1958**, *76*, 673.

Acknowledgment. We are indebted to Dr. J. H. Burchenal and Thomson Pancoast for the tumor growth inhibition data and to Dr. Frank Field of the Mass Spectrometric Biotechnology Research Resource of Rockefeller University for the mass spectra. We also thank Iris Wempen for providing the FIAC. This investigation was supported in part by funds from the National Cancer Institute (Grants CA-08748 and CA-18601) and the National Institute of Allergy and Infectious Disease (Grant AI18600) (R.F.S.), by the National Institutes of Health, and by a grant from the Veterans Administration (R.F.S.).

Registry No. 1, 69123-90-6; **3**, 83546-42-3; **4**, 56632-83-8; **5**, 95740-11-7; **6**, 95740-12-8; **7**, 95740-13-9; **8**, 95740-14-0; **9**, 95740-15-1; **10**, 95740-16-2; **11**, 95740-17-3; **12**, 95740-18-4; **13**, 95740-19-5; **14**, 95740-20-8; **15**, 95740-21-9; **16**, 69123-98-4; **17a**, 95740-22-0; **17b**, 95740-23-1; **18a**, 95740-24-2; **18b**, 95740-25-3; **19a**, 85714-55-2; **19b**, 95740-26-4; thymidine kinase, 9002-06-6; ethyl acrylate, 140-88-5; ethylene, 74-85-1; (trimethylsilyl)acetylene, 1066-54-2.

Isoxazoles with Antipicornavirus Activity†

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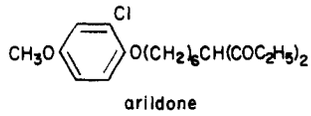
The synthesis and evaluation of a series of 3,5-disubstituted isoxazoles as antipicornavirus agents have led to the discovery of several compounds effective in vitro against rhinovirus type 2 and poliovirus type 2. Compound **32** was found more effective than 4′,6-dichloroflavan against both viruses and was evaluated orally in mice infected intracerebrally with polio-2. At 31 mg/kg bid, compound **32** showed a 53% survival rate as compared to 22% for the nonmedicated animals.

Picornavirus infections are among the most common viral infections in man. This family of viruses consists of the rhinoviruses, of which there are over 120 serotypes, and the enteroviruses comprised of polio, Coxsackie A and B, ECHO, and five unclassified enteroviruses, including hepatitis A. Rhinoviruses are responsible for approximately 50% of the common cold infections and cause mild localized infections of the upper respiratory tract. The enteroviruses cause a broad spectrum of clinical illnesses ranging from mild upper respiratory ailments to more severe diseases such as aseptic meningitis, myocarditis, and poliomyelitis. Generally, enterovirus infections of the pediatric population result in greater morbidity and mortality.[1] Recent studies have shown that relatively mild pediatric infections can result in long-term neurological sequelae.[2]

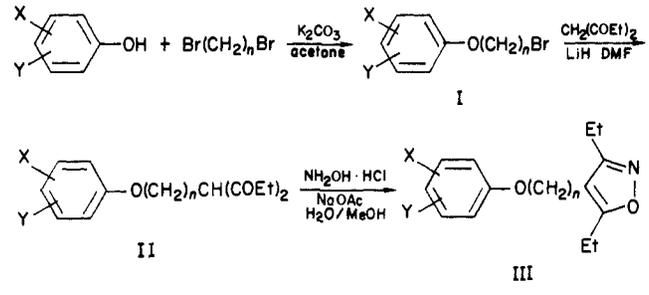
In view of the lack of chemotherapeutic agents available for the treatment of picornavirus infections, we initiated a program directed towards the discovery of compounds active against this class of viruses. We initially examined compounds related to arildone, since this compound, in



arildone

addition to exhibiting in vitro activity against herpesvirus,[3] was effective against poliovirus[4] and had demonstrated efficacy when administered orally to mice infected intracerebrally with polio-2.[5] Arildone, however, was only marginally effective against the rhinoviruses, and since the objective was to synthesize an agent with broad spectrum activity against the picornaviruses, we began screening related compounds. As a result of this screening, the isoxazoles III possessing in vitro activity against both

Scheme I. Synthesis of 3,4,5-Trisubstituted Isoxazoles



† Presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Las Vegas, NV, October 1983.

(1) Lepow, M. L. *Pediatrics* **1978**, *62*, 267–269.
(2) Sumaya, C. V.; Corman, L. I. *Pediatr. Infect. Dis.* **1982**, *1*, 151 (1982).
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(4) Caliguiri, L. A.; McSharry, J. J. *Abstr. Am. Soc. Microbiol.* **1975**, *79*, 259.
(5) McKinlay, M. A.; Miralles, J. V.; Brisson, C. J.; Pancic, F. *Antimicrob. Agents Chemother.* **1982**, *22*, 1022.
(6) Micetich, R. G. *Can. J. Chem.* **1970**, *48*, 2006.
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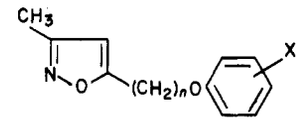
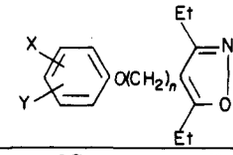
Table I. Intermediate Nitriles and Acids[a]



compd	n	X	yield, %	mp, °C	recrystn solvent[b]	formula[c]
1	7	4-CN	31.2	56	B	$C_{18}H_{22}N_2O_2$
2	7	4-COOH	94.6	129–130	A	$C_{18}H_{23}NO_4$
3	6	4-CN	48.8	54	C	$C_{17}H_{20}N_2O_2$
4	5	4-CN	43.1	60–51	C	$C_{16}H_{18}N_2O_2$
5	5	4-COOH	78	151	A	$C_{16}H_{19}NO_4$
6	7	3-COOH	89.5	95–96	A	$C_{18}H_{23}NO_4$
7	8	4-CN	39.9	60	C	$C_{19}H_{24}N_2O_2$
8	8	4-COOH	89.7	115–116	A	$C_{19}H_{23}NO_4$

[a] All of these compounds were inactive against RV-2 and polio-2. [b] Recrystallization solvents: A = EtOH, B = ether, C = ether–pentane. [c] The elemental analysis (C, H, and N) for all new compounds were within ±0.4% of the theoretical values.

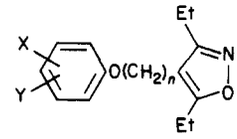
Table II. In Vitro Antipicornavirus Activity of 3,4,5-Trisubstituted Isoxazoles



no.	X	Y	n	mp, °C or bp, °C	% yield	formula[a]	RV-2[b]	polio-2[c]	MTL[d]
9	2-Cl	4-CH_3O	6	*e*	55	$C_{20}H_{28}ClNO_3$	0.8	NA	12
10	H	4-OH	6	106–108[f]	73	$C_{19}H_{27}NO_3$	NA	NA	25
11	H	4-CH_3S	6	*g*	86	$C_{20}H_{29}NO_2S$	NA	0.6	12.5
12	2-CF_3	H	6	*g*	75	$C_{20}H_{26}F_3NO_2$	3.1	NA	6.2
13	3-I	H	6	*e*	59	$C_{19}H_{26}INO_2$	6.3	NA	12.5
14	2-NO_2	4-CH_3O	6	200–205 (0.02 mm)	75.8	$C_{20}H_{28}N_2O_5$	0.3	1.5	6.0
15	H	4-Br	6	190–195 (0.001 mm)	73.7	$C_{19}H_{26}BrNO_2$	NA	NA	6.0
16	2-Cl	4-F	6	*g*	74	$C_{19}H_{25}ClFNO_2$	3.1	NA	12.5
17	2-F	H	6	*g*	79	$C_{19}H_{26}FNO_2$	3.1	NA	6.2
18	3,4-OCH_2O		6	*g*	42	$C_{20}H_{27}NO_4$	NA	NA	6.2
19	2-Cl	6-Cl	6	*g*	90	$C_{19}H_{27}Cl_2NO_3$	1.6	5.9	12.0
20	H	4-CH_3O	6	*g*	91	$C_{20}H_{29}NO_3$	3.1	NA	6.2
21	H	4-$COOC_2H_5$	6	*g*	75	$C_{22}H_{31}NO_4$	0.1	0.5	6.2
22	H	4-$COOC_2H_5$	7	*g*	40	$C_{23}H_{33}NO_4$	0.05	0.4	12.0
23	H	4-$COOC_2H_5$	8	*e*	64	$C_{24}H_{35}NO_4$	0.4	NA	12.5
24	2-CH_3O	6-CH_3O	6	*g*	75	$C_{21}H_{31}NO_4$	3.1	NA	6.2
25	2-Br	4-CH_3O	6	*g*	85	$C_{20}H_{28}BrNO_3$	0.4	NA	6.0
26	2-$COOCH_3$	4-CH_3O	6	*h*	82	$C_{22}H_{31}NO_5$	NA	2.9	12.0
27	2-COOH	4-CH_3O	6	*i*	76[k]	$C_{21}H_{29}NO_5$	NA	NA	12.5
28	H	4-COOH	6	91–93[j]	51[l]	$C_{20}H_{27}NO_4$	NA	NA	3.1
29	2-Cl	4-$COOCH_3$	6	*h*	86	$C_{21}H_{18}ClNO_4$	0.4	0.61	1.6
30	2-Br	4-$COOCH_3$	6	*g*	36	$C_{21}H_{18}BrNO_4$	0.4	2.2	12.5

[a] The elemental analysis (C, H, and N) for all new compounds were within ±4% of the theoretical values. [b] Rhinovirus type 2. [c] Poliovirus type 2. [d] Maximum testable level. [e] Pure sample obtained by column chromatography or silica gel and eluted with hexane–ethyl acetate (4:1). [f] Recrystallized from CH_2Cl_2. [g] Eluted with hexane–ethyl acetate (3:1). [h] Eluted with hexane–ethyl acetate (1:1). [i] Eluted with ethyl acetate–hexane (3:1). [j] Recrystallized from ethyl acetate. [k] Prepared from **26**. [l] Prepared from **21**.

rhinovirus type 2 (RV-2) and poliovirus type 2 (polio-2) were discovered. Consequently, a variety of related 3,4,5-trisubstituted as well as some 3,5-disubstituted isoxazoles were prepared.



III

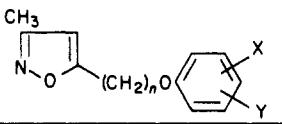
Chemistry. The 3,4,5-trisubstituted isoxazoles were prepared from the corresponding diketones[1] according to Scheme I. In the majority of cases the products were purified by column chromatography as oils.

The synthesis of the 3,5-disubstituted isoxazoles is shown in Scheme II. Treatment of 3,5-dimethylisoxazole with either *n*-butyllithium or LDA at −70 °C,[4] followed by alkylation with bromide I, gave IV in 65% yield. Esters VII, with the exception of the *tert*-butyl ester **41**, could not be prepared by direct alkylation due to the formation of side products as a consequence of the interaction of the ester group with the carbanion of dimethylisoxazole. The hydrolysis of the nitrile V gave the acids VI, which were esterified or amidated.

Biology. Several trisubstituted isoxazoles were evaluated against RV-2 in vitro by the plaque reduction method described in the Experimental Section. The initial lead compound (**9**), derived from arildone, exhibited an MIC of 0.8 μg/mL (Table II). Replacing the chloro group in **9** with a bromo (**25**) or nitro (**14**) group increased the activity twofold, as did replacing the methoxy group with a carbomethoxy group (**29** and **30**). By far the greatest enhancement of activity was produced by the 4-carboethoxy homologues **21**–**23**, with **22** exhibiting an MIC of 0.05 μg/mL against RV-2. Compounds **21** and **22** also demonstrated appreciable activity against poliovirus type 2.

Concurrently, a series of 3,5-disubstituted isoxazoles was also synthesized (Tables I and III). As in the previous series, the two most active compounds contained the 2-

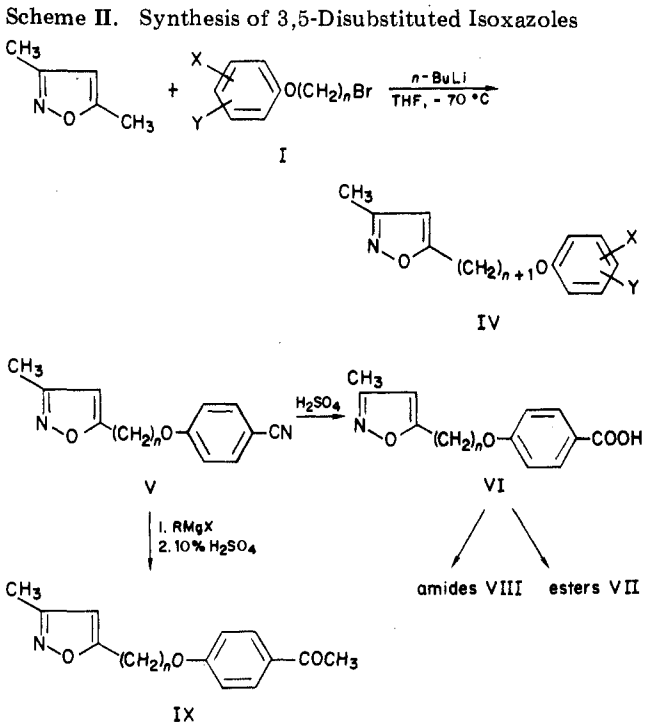
Table III. In Vitro Antipicornovirus Activity of 3,5-Disubstituted Isoxazoles



no.	X	Y	n	mp, °C	% yield	formula[a]	RV-2[b]	polio-2[c]	MTL[d]
31	2-Cl	4-CH_3O	8	35.5[e]	70	$C_{19}H_{26}ClNO_3$	0.16	0.65	12.5
32	2-Cl	4-CH_3O	7	45–46[e]	59	$C_{18}H_{24}ClNO_3$	0.04	0.05	12.5
33	2-Cl	4-CH_3O	6	*f*	53	$C_{17}H_{22}ClNO_3$	0.8	0.3	12.5
34	2-Cl	4-CH_3O	5	*f*	65	$C_{16}H_{22}ClNO_3$	0.4	0.2	12.5
35	H	4-BzO-	7	82–83[g]	68	$C_{24}H_{29}NO_3$	NA		12.5
36	H	4-$COOC_2H_5$	4	61[h,i]	96	$C_{17}H_{21}NO_4$	3.1	NA	6.2
37	H	4-$COOC_2H_5$	5	50–51[i,j]	46	$C_{18}H_{23}NO_4$	0.015	0.6	12.5
38	H	4-$COOC_2H_5$	6	51[h,i]	77	$C_{19}H_{25}NO_4$	0.01	0.8	12.5
39	H	4-$COOC_2H_5$	7	60–61[i,k]	60	$C_{20}H_{27}NO_4$	0.025	0.8	2.5
40	H	4-$COOC_2H_5$	8	49[g,i]	76	$C_{21}H_{29}NO_4$	0.05	NA	12.5
41	H	4-$COOC(CH_3)_3$	7	76[h]	23	$C_{22}H_{31}NO_4$	6.2	1.2	2.5
42	H	4-$COOCH(CH_3)_2$	7	45–46[l]	57	$C_{21}H_{29}NO_4$	0.2	0.9	12.5
43	H	4-$COOCH_3$	7	60[m]	97	$C_{19}H_{25}NO_4$	3.0	0.4	6.2
44	H	4-$COO(CH_2)_2CH_3$	7	54[j]	77	$C_{21}H_{29}NO_4$	0.07	NA	25
45	H	4-$CONHNH_2$	7	121–122[g]	86	$C_{18}H_{25}N_3O_3$	0.64	NA	12.5
46	H	4-$CONH_2$	7	153–154[k]	72	$C_{18}H_{24}N_2O_3$	NA	NA	12.5
47	H	4-CH_3CO	7	69–71[g]	60	$C_{19}H_{25}NO_3$	1.6	0.01	12.5
48	H	4-C_2H_5CO	7	61[g]	58	$C_{20}H_{27}NO_3$	1.7	0.4	6.2
49	H	4-$CON(CH_3)_2$	7	58–59[h]	75	$C_{20}H_{28}N_2O_3$	NA	2.5	3.1
50	H	4-$OCH(CH_3)_2$	7	38[j]	71	$C_{20}H_{29}NO_3$	0.70	0.1	12.5
51	H	4-$CH(CH_3)_2$	7	*n*	71	$C_{20}H_{29}NO_2$	1.0	0.04	12.5
52	H	3-$COOC_2H_5$	7	16–18[h]	63	$C_{20}H_{27}NO_4$	1.6	NA	12.5
53	H	4-NO_2	7	58[o]	95	$C_{17}H_{22}N_2O_4$	NA	1.5	1.6
54	2-Cl	4-CH_3	7	p	88	$C_{18}H_{24}ClNO_2$	1.2	0.8	6.2
55	2-NO_2	4-CH_3O	7	51[j]	75	$C_{18}H_{24}N_2O_5$	0.3	0.2	3.1
56	H	H	7	60[o]	82	$C_{17}H_{23}NO_2$	1.2	1.7	6.2
57	H	4-Cl	7	81[o]	69	$C_{17}H_{22}ClNO_2$	1.5	0.3	3.1

[a] The elemental analysis (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] Sample recrystallized from isopropyl acetate. [f] Pure sample obtained by column chromatography on silica gel and eluted with ether–hexane (1:1). [g] Sample recrystallized from ethanol. [h] Recrystallized from hexane. [i] Prepared by esterification of the corresponding acid. [j] Recrystallized from ether–pentane. [k] Recrystallized from methanol. [l] Recrystallized from pentane. [m] Recrystallized from methanol–hexane. [n] Pure sample obtained by column chromatography and eluted with hexane–ether (9:1). [o] Recrystallized from ether. [p] Pure sample obtained by column chromatography and eluted with ether.

Scheme II. Synthesis of 3,5-Disubstituted Isoxazoles



chloro-4-methoxyphenyl and 4-carbethoxyphenyl moieties. In the former case, optimum activity was obtained with the C-7 chain (**32**) while in the latter series, the C-6 homologue (**38**) was the most active against RV-2. In addition, both compounds were effective against polio-2; however, **32** was significantly more active than **38**. The 2-nitro-4-methoxy homologue (**55**), although quite active in the 3,4,5-trisubstituted series, was less active than **32**.

Table IV. Comparative Evaluation of Compounds **32**, **38**, and 4′,6-Dichloroflavan

compd	MIC, μg/mL RV-2	polio-2	polio-3	echo-9	echo-11
32	0.04	0.05	0.08	0.15	0.04
38	0.01	>0.8			
4′,6-dichloroflavan	0.09	>3.1			

It is interesting to note that some compounds, such as the 4-carbomethoxy **43**, 4-methyl and ethyl ketones **47** and **48**, 4-isopropyl **51**, and 4-chloro homologues were more effective against polio-2 than rhino-2.

Compounds **32** and **38** were screened against several other enteroviruses (Table IV). Despite its high level of activity against RV-2, compound **38** exhibited no activity against polio-2, echo-3, and echo-11, while compound **32** was consistently active against all five viruses.

When administered orally to poliovirus-infected mice, compounds containing a carbethoxy moiety, although very effective in inhibiting virus replication in vitro, had no effect on the course of poliovirus infection in mice. This was most likely due to the metabolic hydrolysis of compounds such as **38** to the inactive acid.[2] Consequently, various modifications of the ester were made including the *tert*-butyl (**41**), isopropyl (**42**), methyl (**43**), and propyl (**44**) esters, none of which demonstrated activity comparable

Table V. Effect of Compound **32** on Plaque Production by 27 Human Rhinovirus Serotypes[a]

rhinovirus serotype	in vitro MIC, μg/mL	rhinovirus serotype	in vitro MIC, μg/mL
1A*	0.29	29	3.10
1B*	0.37	29*	0.33
2*	0.04	31*	0.53
3	0.50	33	0.05
4*	NA	36	0.04
5	5.80	39	0.07
13	1.90	41	NA
14	1.35	49	0.26
15*	0.44	50	0.13
16	0.04	61	0.09
18	1.50	72	NA
21	0.01	75	4.10
22	0.10	86	0.25
		89	0.03

[a] Asterisk indicates seven most common serotypes.

Table VI. Prevention of Poliovirus-Induced Paralysis and Death in Mice Medicated Orally with Compound **32**[a]

dose, mg/kg	% survival[b]	no. of survivors/ total no. of animals
250	93 ± 5.7	28/30
125	73 ± 11.5	22/30
63	67 ± 15.3	20/30
31	53 ± 5.8	16/30
placebo	22 ± 11.5	7/30
nonmed	22 ± 15.3	7/30

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

to that of the ethyl esters. Amides **45**, **46**, and **49** demonstrated only marginal activity. Ketones **47** and **48** were appreciably active against polio-2 but weakly active against RV-2.

Compound **32** was screened against 27 serotypes of rhinovirus and was effective against 24 in the range of 0.01–5.8 μg/mL (Table V).

In Vivo.[10] Compound **32** was tested in mice infected intracerebrally with polio-2 according to the procedure described in the Experimental Section. In the initial test, mice were medicated orally 1 h prior to, 2-h postinfection, and twice daily for 13 days thereafter at doses of 31, 63, 125, and 250 mg/kg bid. The results are shown in Table VI. A dose response was observed upon termination of the test on day 20. At 250 mg/kg there were 93% survivors, while at 125, 63, and 31 mg/kg the corresponding percent survivors was 73, 67, and 53, while the placebo and nonmedicated group showed a 22% survival rate. The MIC for **32**, considered to be the lowest dose that results in significantly increased survival, was determined to be 31 mg/kg bid.

In a related test mice were medicated 1-h postinfection with 250 mg/kg of **32**. At the end of the medication period (14 days postinfection), only 10% and 15% of the placebo and nonmedicated animals were alive, respectively, compared to 70% of the drug-treated animals.

Mode of Action. Mode of action studies on **32**, which will be reported elsewhere, demonstrate that the drug interferes with viral uncoating by reversibly binding to the virus.

Discussion

Compound **32** compared favorably with 4′,6-dichloroflavan (BW 683c) with respect to its activity against several strains of rhinovirus, exhibiting MIC values of 0.1–5.80 μg/mL, against 14 serotypes vs. 0.007–10 for 4,6-dichloroflavan against the same serotypes.[9] In our hands, 4′,6-dichloroflavan was less effective than **32** against RV-2 and inactive against polio-2 (Table IV).

Compound **32** has been shown to be absorbed when administered orally (ig) to mice. Not only has activity been demonstrated against polio-2 in mice but also blood levels were detected by HPLC above the MIC demonstrated in vitro against this virus.

In summation, this series of compounds has exhibited a broad spectrum of activity against both rhinoviruses and enteroviruses and has been found to be orally effective against polio-2. Compound **32** demonstrated a novel virus-specific mode of action, representing an interesting and novel approach to the chemotherapy of picornavirus infections.

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 JEOLCO double-focusing high-resolution mass spectrophotometer by S. Clemans. All of the diketones whose synthesis are not described in this manuscript were previously reported.[3]

In Vitro Plaque Reduction Screens. Compound Preparation. Compounds were solubilized in Me_2SO at 10 mg/mL and serially diluted twofold in Me_2SO until 2000 times the final concentration was reached. A 1:100 dilution was then made into 2X M-199 plus 10% Bobby calf serum and the solution was diluted 1:1 with 1% melted agarose and kept in a 40 °C water bath until needed for overlay. In the case of rhinoviruses, the medium-agarose was adjusted to a final concentration of 15 μg/mL DEAE-dextrose and 38 mM $MgCl_2$ before storing.

Infection. Maintenance medium was aspirated from day-old 90% Hela (Ohio) cell monolayers and infected with 1.0 mL/well of the appropriate dilution of RV-2 or polio-2 to yield ca. 80 pfu/well. Plates were incubated at least 1 h at 33 °C (37 °C for polio) in a 2% CO_2 atmosphere. The viral inoculum was removed, and cells were overlaid in duplicate with the drug–agarose medium solutions. Virus-infected controls and uninfected cell controls (Me_2SO only) were run at the same time. Virus was allowed to replicate at 33 °C in a 2% CO_2 atmosphere for 3 days. Cells were fixed with formaldehyde solution and stained with crystal violet. Plaques, appearing as clear areas of cell destruction, were counted. The concentration of compound that resulted in a 50% reduction in the number of plaques was determined and was termed the minimal inhibitory concentration (MIC). The maximum testable level (MTL) was the concentration of drug below which no cytotoxic effects were observed.

In Vivo Test. Mouse Infection with Polio-2. ICR Buckberg mice, weighing 18–21 g each, were inoculated in the left cerebral hemisphere with 0.03-mL volumes of tenfold dilutions of plaque-purified polio-2 (MEF strain). Ten animals were medicated orally at 1-h postinfection. Animals were observed daily over a 21-day period for the development of flaccid limb paralysis, as well as death.

General Methods of Synthesis. Preparation of 3,4,5-Trisubstituted Isoxazoles. Ethyl 4-[[6-(3,5-Diethyl-4-isoxazolyl)hexyl]oxy]benzoate (21). A mixture of 10.8 g (0.0287 mol) of ethyl 4-[[8-oxo-7-(1-oxypropyl)decyl]oxy]benzoate,[3] 2.47 g (0.0356 mol) of hydroxylamine hydrochloride, 4.49 g (0.0330 mol) of $NaOAc{\cdot}3H_2O$ in 250 mL of C_2H_5OH, and 50 mL of H_2O was heated to reflux for 18 h. The mixture was concentrated in vacuo and the residue was extracted with CH_2Cl_2 and H_2O. After drying, the organic layer was concentrated in vacuo and the oily residue

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(10) Complete details of this in vivo study will be reported elsewhere.

purified by column chromatography with 75% C_6H_{14} and 25% EtOAc to give 8 g (74.7%) of a straw-colored oil. Anal. ($C_{22}H_{31}NO_4$) C, H, N.

3,5-Disubstituted Isoxazoles. 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-3-methylisoxazole (32). A solution of 2.91 g (0.03 mol) of 3,5-dimethylisoxazole in 70 mL of THF was cooled to −70 °C, at which point 18.8 mL of 1.55 M *n*-butyllithium (0.03 mol) in C_6H_{14} was added under N_2 over a 10-min period. The solution was stirred for an additional 30 min at −70 °C and then 9.6 g (0.03 mol) of 1-bromo-6-(2-chloro-4-methoxyphenoxy)hexane[3] in 15 mL of THF was added dropwise over a period of 1 h. The mixture was allowed to warm to room temperature and then left overnight. The mixture was concentrated to dryness in vacuo and the resulting oil crystallized on standing and was recrystallized from a mixture of $(C_2H_5)_2O$–C_6H_{14} to give 6 g of a white solid (59.2%); mp 45–46 °C. Anal. ($C_{18}H_{24}ClNO_3$) C, H, N.

4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzoic Acid (2). A solution of 10.0 g (0.033 mol) of nitrile 1 in 60 mL of 20% aqueous HCl and 60 mL of glacial HOAc was heated to reflux for 30 h. After the solution cooled, the resulting solid was collected by filtration and dried to give a white solid, which was recrystallized from C_2H_5OH; 8.5 g (94.6%); mp 129–130 °C. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

Ethyl 4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzoate (39). A solution of 8.5 g (0.027 mol) of 1 in 90 mL of ethanol and 1.1 mL of concentrated H_2SO_4 was heated to reflux for 6 h. On cooling, solid separated and was collected. Recrystallization from ethanol gave 8.0 g (92%); mp 60–61 °C. Anal. ($C_{20}H_{27}NO_4$) C, H, N.

3-Methyl-5-[7-(4-Carboxamidophenoxy)heptyl]isoxazole (46). To a solution of 4.47 g (0.015 mol) of nitrile 2 in 18 mL of 95% EtOH and 6 mL of 30% H_2O_2 was added 0.6 mL of 6 N NaOH at ambient temperature.[6] The temperature rose to 50 °C with much foaming. Cooling was necessary to maintain a temperature of 40–50 °C and then the solution was kept at this temperature for 4 h and left at room temperature overnight. The solution was neutralized with 34 mL of 5% H_2SO_4 and chilled. The resulting solid was collected, washed with cold EtOH, and dried. The material was recrystallized from CH_3OH to give 4.4 g (72%); mp 153–154 °C. Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

1-[4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]phenyl]ethanone (47). To 1.092 g (0.045 mol) of magnesium was added dropwise 2.825 mL (0.045 mol) of methyl iodide in 15 mL of ether. The addition was carried out at such a rate that a gentle reflux was maintained. After the mixture was stirred for $^1/_2$ h, an additional 0.5 mL of methyl iodide was added until all the magnesium had reacted. The solution was then heated to reflux for $^1/_2$ h. After the solution cooled, 8.46 g (0.03 mol) of 4-[[7-(3-methyl-5-isoxazolyl)heptyl]oxy]benzonitrile (1) in 12 mL of benzene was added dropwise. After the addition was complete the mixture was refluxed for 3 h. To the cooled mixture was added 60 mL of a saturated NH_4Cl solution with stirring. The supernatant liquid was removed by decantation and the residual gum stirred with 50 mL of dilute HCl for 1 h. The aqueous solution was heated to reflux for 1 h during which time a gum separated, which eventually solidified. The solid was collected and dried. Recrystallization from C_2H_5OH gave 5.35 g (60%); mp 69–71 °C. Anal. ($C_{19}H_{25}NO_3$) C, H, N.

1,1-Dimethylethyl 4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzoate (41). Compound 41 was prepared in 23% yield by the general procedure described, after recrystallization from C_6H_{14};mp 76 °C. Anal. ($C_{22}H_{31}NO_4$) C, H, N.

Ellipticine Derivatives with an Affinity to the Estrogen Receptor, an Approach to Develop Intercalating Drugs with a Specific Effect on the Hormone-Dependent Breast Cancer

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In order to obtain breast tumor directed agents, we have prepared mixed compounds using estradiol or (*E*)-clomiphene as specific vectors for the breast tissue and a DNA intercalator from the ellipticine series as the cytotoxic agent. Among the newly synthesized ellipticine derivatives, only the 2-[3-aza-5-(3,17β-dihydroxy-1,3,5-estratrien-17α-yl)-4-oxopentamethylene]ellipticinium bromide (24) shows the desired properties, DNA intercalation and affinity for estrogen receptor. Competition experiments with estradiol on the hormone-dependent human MCF-7 breast cancer cell line demonstrate that a transport by the estrogen receptor system is not involved in the antitumor activity of derivative 24.

Clinical use of most antitumor drugs is limited by their high toxicity for fast-growing cells in healthy tissues. With the object of overcoming this problem, several cytotoxic agents, such as nitrogen mustards,[1,2] intercalating drugs,[3-5] toxins,[6-8] have been coupled to carriers exhibiting some selectivity toward the tumors themselves[2,5] or to the tissues from which these tumors derive.[1,9] This strategy is aimed at concentrating cytotoxic agents into cells bearing binding sites for the carrier.

The growth of endometrium and of nearly 30% of breast cancers is stimulated by estrogens. Antiestrogens antagonize estrogen effects, and clinical studies have shown that these compounds could be used to treat estrogen-dependent tumors with some success. The mechanism of action of estrogens[10] and probably antiestrogens[11,12] is

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