proposed by Rubin et al.[23] Body temperature was measured rectally by means of a thermocouple metal probe. Groups of ten mice were housed the evening before the experiment with a 9-h fasting period but free access to water until 2 h before testing sessions. All the compounds were tested at the screening dose of 25 mg/kg, and the active ones at this dose were subsequently tested at lower doses, in order to obtain an ED_{50} by "eye-fit" linear plots on semilogarithmic paper. The compounds were administered 30 min before ip injection of 2.5 mg/kg of reserpine (dissolved in 0.5% acetic acid in a volume of 0.05 mL/10 g of body weight). The parameters considered were read blind 1 (blepharospasm) and 4 h (hypothermia) after reserpine treatment.

Pentylenetetrazole Antagonism. All the compounds were tested for their effect on the maximal extensor seizures induced by pentylenetetrazole given by ip injection at a dose of 130 mg/kg in a volume of 0.1 mL/10 g of body weight. This dose of pentylenetetrazole induces clonic and flexor-extensor tonic convulsions in 100% of the control animals. The test compounds were administered at different dosages 30 min before treatment with pentylenetetrazole, starting from the screening dose of 50 mg/kg, in order to obtain the dose-response curves. Groups of ten animals were used for each dose tested and for controls. From the dose-response curves obtained the dose was calculated, which prevents the onset of flexor-extensor tonic convulsions in 50% of the treated animals (ED_{50}).

Orientative Acute Toxicity. This information (ca. LD_{50}) was obtained in the course of the Irwin's test,[24] performed on all the tested compounds in order to have their symptomatologic profile. All the compounds were tested at least at six dose levels (25, 50, 100, 200, 400, and 800 mg/kg) on four animals for dose with careful observation for 60 min after treatment. Deaths occurring in 7 days after drug administration were registered.

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Easily Hydrolyzable, Water-Soluble Derivatives of (±)-α-5-[1-(Indol-3-yl)ethyl]-2-methylamino-Δ^2-thiazolin-4-one, a Novel Antiviral Compound

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The preparation of a series of indole *N*-acyl and *N*-carbamic esters of (±)-α-5-[1-(indol-3-yl)ethyl]-2-methylamino-Δ^2-thiazolin-4-one (**1**) is reported. These derivatives were synthesized as potential water-soluble precursors of the antiviral thiazolinone **1**, for evaluation by intranasal administration against influenza and other respiratory infections caused by viruses. Salts of the basic carbamic esters (**16–19**) possess the required water solubility, undergo rapid hydrolysis and decarboxylation at pH values greater than 6, and have high activity against influenza A_2 and Coxsackie B_1 viruses in vitro. In influenza A_2 infected ferrets a representative ester (**16**) reduced the severity and duration of disease symptoms and reduced nasal wash virus titres but caused local irritancy.

As a consequence of the intracellular replication of viruses and close integration of the biochemistry of virus replication and host cell metabolism, most compounds which have been shown to inhibit virus multiplication also interfere with some processes involved in the metabolism of uninfected cells. This anti-cell-activity can result in toxic effects in animals, such as immunosuppression and teratogenicity. However, when such compounds are administered systemically only a small proportion of the administered drug actually reaches the site of virus infection, which is often restricted to a particular tissue. Thus, for many viral diseases, significantly improved therapeutic ratios can be achieved by administration of the drug directly to the site of infection. This approach has, for example, been used successfully in the ocular treatment of herpes simplex keratitis with the nucleoside analogues 5-iodo-2′-deoxyuridine and 9-(β-D-arabinofuranosyl)adenine, which have activity against DNA viruses.

Infections with influenzas and rhinoviruses, which are RNA viruses, occur predominantly in the epithelium of the upper respiratory tract of man and should also be amenable to treatment with topically administered drugs. A major difficulty with intranasal administration is, however, that the mechanisms for clearance of both soluble and particulate foreign materials from the upper respiratory tract are extremely efficient. Only highly active compounds with high water solubility would therefore be expected to be sufficiently biologically available and even then many doses may be required daily. Provided that this

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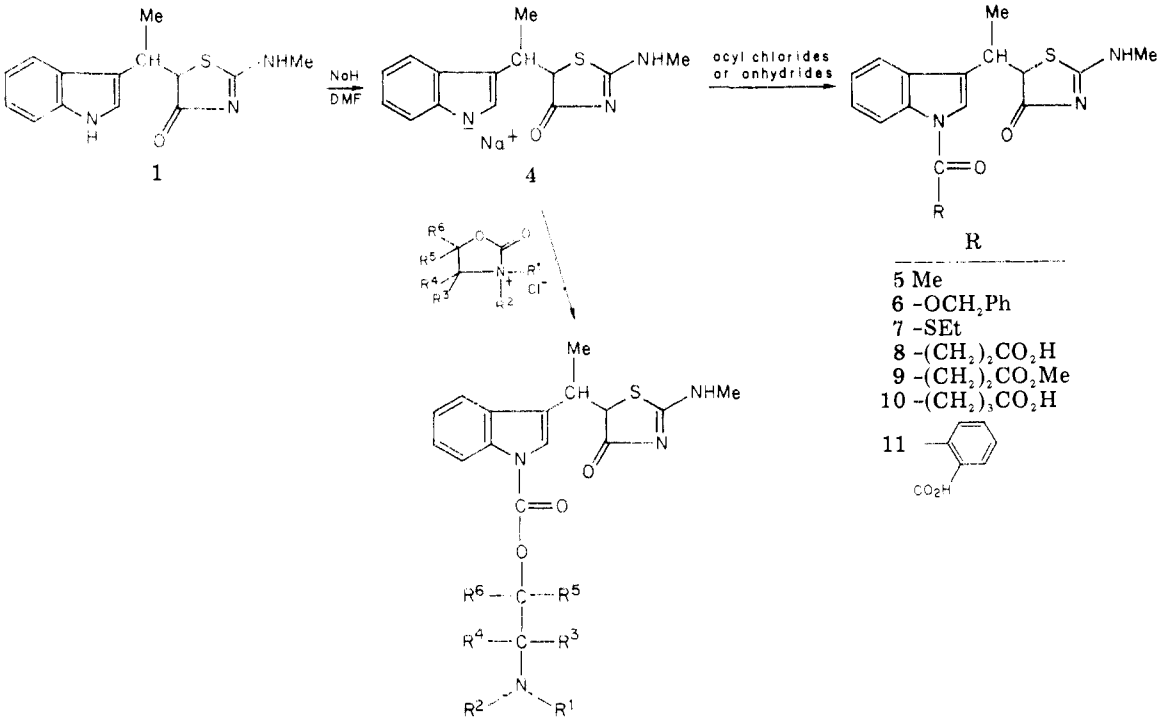
Scheme I

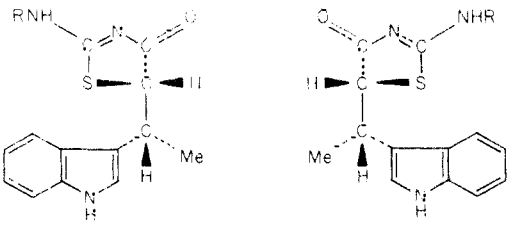


	R¹	R²	R³	R⁴	R⁵	R⁶
16	Me	Me	Me	Me	H	H
17	Me	Me	H	H	Me	Me
18	-(CH2)2O(CH2)2-		Me	Me	H	H
19	Me	Me	H	H	Me	H

does not result in the concomitant oral ingestion of the compound in quantities approaching those required for oral activity, the therapeutic ratio achieved using intranasal administration should, nonetheless, be higher than that achieved with systemic dosing.

In previous publications, we have described the synthesis,[1] stereochemistry,[1,2] and anti-RNA-virus and antibacterial activity[3] of (5*S*)-5-[(1*R*)-1-(indol-3-yl)ethyl]-2-methylamino-Δ^2-thiazolin-4-one, the thiazolinone analogue of indolmycin. This compound has a spectrum of in vitro antimicrobial activity which is of considerable interest for the treatment of viral infections of the upper respiratory tract but, because of its very low water solubility, it is unsuitable for administration by the intranasal route.

Since more water-soluble analogues (**2** and **3**) of the racemic thiazolinone **1** have reduced biological activity,[3]



1, R = CH_3; 2, R = CH_2CH_2OH; 3, R = $CH_2CH_2NMe_2 \cdot HCl$

our attention was directed toward the synthesis of water-soluble derivatives of **1** which would rapidly release the thiazolinone **1** in vivo.

In this publication, the preparation of two series of such compounds and their activity against influenza A_2 and Coxsackie B_1 viruses in tissue culture and against influenza A_2 in ferrets are described (Scheme I).

Chemistry. The indolyl anion **4** was generated from the thiazolinone **1** using sodium hydride in *N*,*N*-dimethylformamide. This anion (**4**) was reacted in situ with acetyl chloride, benzyl chloroformate, ethyl thiochloroformate, and 3-(carbomethoxy)propionyl chloride, yielding the *N*-acyl derivatives **5**–**7** and **9**, respectively, and also with cyclic anhydrides, yielding the carboxylic acids **8**, **10**, and **11**. Sodium salts of the carboxylic acids **8**, **10**, and **11** have high water solubility and are stable in aqueous solutions at neutral pH. For the synthesis of the basic carbamic esters **16**–**19**, the anion **4** was reacted with the 2-oxooxazolidinium salts **12**–**15**, prepared from branched-chain dialkylaminoalkanols and phosgene.[4] The basic ester **16** was converted to its quaternary methiodide **20** using methyl iodide in tetrahydrofuran.

Although the hydrochloride salts of the basic carbamic esters **16**–**19** are highly water soluble, the esters undergo rapid hydrolysis and decarboxylation in unbuffered aqueous solutions at pH >6, yielding the thiazolinone **1**. Hydrolysis of the carbamic ester linkage is facilitated by achimeric assistance from the nitrogen lone pair of the free base, since under similar conditions the neutral carbamic ester **6** and the quaternary derivative **20** are not hydrolyzed.

Antiviral Activity. Acylation of the indole nitrogen of (±)-α-5-[1-(indol-3-yl)ethyl]-2-methylamino-Δ^2-thiazolin-4-one (**1**), as in compounds **5**–**9**, **11**, and **20**, eliminates antiviral activity. Although exact measurements of relative rates of hydrolysis have not been made, it is apparent that only compounds **10** and **16**–**19**, which undergo facile hydrolysis, have activity in vitro (Table I). The rapidly hydrolyzed basic carbamic esters **16**–**19** are the most active compounds prepared, although, as would be expected if they are acting as precursors of the antiviral thiazolinone

Table I. Antiviral Activity in Tissue Culture

	influenza A_2 in MDCK cells			Coxsackie B_1 in HeLa cells		
compd[a]	inhibn zone, mm	PDD_{50}, μg/mL	MCC, μg/mL	inhibn zone, mm	PDD_{50}, μg/mL	MCC, μg/mL
1	30	0.5	3	29	5	30
8	0	>100	>100	0	100	>100
10	24	5	100	20	10	>100
16	30	0.3	3	35	20	100
17	25	0.3	3	21	5	30
18	26	2	30	24	5	100
19	31	0.5	10	25	20	100
Ribavirin[b]	30	2	>100			
HBB[c]				25	20	>100

[a] Compounds 5-7, 9, 11, and 20 were also tested but had no antiviral activity or cytotoxicity at concentrations up to 100 μg/mL. [b] Ribavirin = 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. [c] HBB = 2-(α-hydroxybenzyl)benzimidazole.

Table II. Antiviral Activity in Influenza A/Port Chalmers/1/73 Infected Ferrets

	mg/kg per dose[a]	% body wt change: days 0 to +7	rectal temp[b] >39.5 °C: day +1	+2	+3	+4	>40.0 °C: day +1	+2	+3	+4	nasal wash virus titre, HAU/mL: day +2	+3	+4	serum antibody[c] to influenza virus Port Chalmers: pre-infect	10 days post-infect
untreated		0	0	4	0	0	0	3	0	0	32	<4	<4	80	320
		+1.5	0	3	4	0	0	1	1	0	16	8	<4	40	480
		−0.7	0	4	0	0	0	2	0	0	32	<4	<4	40	480
Ribavirin	7	−4.5	0	0	0	1	0	0	0	0	<4	<4	<4	30	480
	14	−22.2	0	0	0	0	0	0	0	0	<4	<4	<4	20	*d*
	13	−20.1	0	0	0	0	0	0	0	0	<4	12	<4	30	*e*
16·HCl	8	−0.4	0	1	1	0	0	0	1	0	<4	<4	<4	30	240
	13	−3.3	0	2	1	0	0	0	0	0	<4	<4	<4	40	480
	16	−3.2	0	2	2	2	0	0	1	0	<4	<4	<4	30	960

[a] Based on day 0 body weight and 0.2 mL of a 50 mg/mL solution. [b] Number of times the temperatures were exceeded. [c] Reciprocal of the highest dilution of serum giving 50% inhibition of hemagglutination. [d] Ferret died day +10. [e] Ferret died day +9.

1, in no case is the activity of 1 significantly exceeded. Hydrolysis of the glutaryl derivative **10** would be expected to occur more readily than hydrolysis of the succinyl derivative **8**, since anchimeric assistance from the terminal carboxylic acid grouping would involve six- and five-membered cyclic intermediates for **10** and **8**, respectively.[5] The fact that **10** has substantial antiviral activity in vitro, whereas **8** does not, is therefore also consistent with the proposal that the biologically active *N*-acyl derivatives **10** and **16–19** are acting as water-soluble precursors of **1**. Small variations in the relative activities of **10** and **16–19** and the parent thiazolinone **1** in the influenza A_2/MDCK and Coxsackie B_1/HeLa cell plaque reduction assays may be attributable to the different incubation conditions employed in the two systems.

A 50 mg/mL solution of (±)-α-5-[1-[1-[[(2-dimethylamino-2-methylpropyl)oxy]carbonyl]indol-3-yl]ethyl]-2-methylamino-Δ^2-thiazolin-4-one (**16**) hydrochloride, a representative carbamic ester, was administered intranasally to influenza A_2 infected ferrets. The antiviral activity observed was compared with that of Ribavirin administered in the same manner (Table II). **16** induced slight weight loss in smaller ferrets during the treatment period, and the infected animals suffered some discomfort and resisted when dosed. This reaction, which is attributed to local irritancy, was not, however, severe enough to require termination of dosing. During Ribavirin treatment, weight loss was much more pronounced and one animal died on the 9th and one on the 10th day after commencement of dosing. **16** reduced virus excretion in the nasal passages of all animals treated to below levels detectable by the technique used. Ribavirin behaved similarly, although virus was detected in one animal on the 3rd day after infection. The number of high-temperature readings, i.e., temperature >40.0 or >39.5 °C, recorded for animals treated with **16** and, to a greater extent, for those treated with Ribavirin was markedly reduced in comparison with the number for untreated controls. Virus replication in those ferrets treated with **16** was adequate for the animals to mount a normal serum antibody response.

Since the thiazolinone **1**, in addition to possessing activity against influenza A strains, has activity against other RNA viruses such as Coxsackie and rhinoviruses and also possesses antibacterial activity against staphylococcus and streptococcus strains,[3] water-soluble precursors such as the carbamic esters **16–19** may have considerable potential for the topical treatment of respiratory tract infections involving these organisms. The irritancy observed upon intranasal administration of **16** to influenza-infected ferrets could, however, limit progression of these compounds. Investigation of the precise effects of **16** on respiratory tissue is presently being carried out.

Experimental Section

Melting points were determined using a Reichert hot-stage apparatus. IR spectra were recorded with a Perkin-Elmer 157 spectrometer. ^1H NMR spectra were determined for solutions in deuteriated solvents at 60 MHz with a Varian EM 360 spectrometer (tetramethylsilane as internal standard). Elemental analyses were performed by the microanalytical laboratory of Beecham Pharmaceuticals. For chromatographic separations, silica gel refers to Kieselgel H, Type 60 (E. Merck, Darmstadt). All evaporations were carried out under reduced pressure with a rotary evaporator.

Reaction of (±)-α-5-[1-(Indol-3-yl)ethyl]-2-methylamino-Δ²-thiazolin-4-one (1) with Acyl Chlorides. Sodium hydride (0.2 g, 0.008 mol) was added to a stirred solution of the thiazolinone 1 (1.08 g, 0.004 mol) in dry *N,N*-dimethylformamide (10 mL) under nitrogen. After 1 h, either acetyl chloride, benzyl chloroformate, ethyl thiochloroformate, or 3-(carboxymethyl)-propionyl chloride (0.004 mol) was added, and stirring was continued for 18 h. Water (100 mL) was then added, and the aqueous solution was extracted with ethyl acetate (3 × 100 mL). The ethyl acetate solutions were washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated, yielding the crude acylated products **5**–**7** and **9**.

(±)-α-5-[1-[1-(3-Carboxymethylpropionyl)indol-3-yl]-ethyl]-2-methylamino-Δ²-thiazolin-4-one (9): 0.48 g, 30%; mp 191–193 °C (from ethyl acetate); IR ν_{max} (Nujol) 3210 (NH), 1732 (C=O), 1710 (C=O), 1680 (C=O), 1580 (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.31 (d, 3, *J* = 6.8 Hz, $CHCH_3$), 2.80 (s, 0.8) and 2.98 (d, 2.2, *J* = 5 Hz) ($NHCH_3$), 2.40–3.10 (m, partially obscured, 2, CH_2), 3.39 (t, 2, *J* = 6 Hz, CH_2), 3.69 (s, 3, OCH_3), 3.92 (m, 1, $CHCH_3$), 4.92 (d, 1, *J* = 3.2 Hz, CHS), 7.20–8.70 (m, 5, aromatic), 9.48 (br s, 1, $NHCH_3$). Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N.

The other three *N*-acyl derivatives (**5**–**7**) were chromatographed on silica gel, eluted with 2% methanol in ethyl acetate prior to recrystallization.

(±)-α-5-[1-(1-Acetylindol-3-yl)ethyl]-2-methylamino-Δ²-thiazolin-4-one (5): 0.52 g, 42%; mp 214–216 °C (from methanol/ethyl acetate); IR ν_{max} (Nujol) 3250 (NH), 1708 (C=O), 1680 (C=O), 1580 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.25 (d, 3, *J* = 7 Hz, $CHCH_3$), 2.60 (s, 3, CH_3CO), 2.82 (s, 0.5) and 2.89 (s, 2.5) ($NHCH_3$), 3.75 (m, 1, $CHCH_3$), 4.65 (d, 1, *J* = 3 Hz, CHS), 7.10–8.5 (m, 5, aromatic), 9.17 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{16}H_{17}N_3O_2S$) C, H, N.

(±)-α-5-[1-[1-[(Benzyloxy)carbonyl]indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (6): 0.58 g, 36%; mp 184–186 °C (from ethyl acetate); IR ν_{max} (Nujol) 1725 (C=O), 1692 (C=O), 1620 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.19 (d, 3, *J* = 6.6 Hz, $CHCH_3$), 2.80 (s, 0.8) and 2.87 (s, 2.2) ($NHCH_3$), 3.70 (m, 1, $CHCH_3$), 4.63 (d, 1, *J* = 3 Hz, CHS), 5.50 (s, 2, CH_2), 7.20–8.20 (m, 10, aromatic), 9.20 (br s, 1, D_2O exchangeable, $NHCH_3$). Anal. ($C_{22}H_{21}N_3O_3S$) C, H, N.

(±)-α-5-[1-[1-[(Ethylthio)carbonyl]indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (7): 0.43 g, 30%; mp 203–204 °C (from ethyl acetate); IR ν_{max} (Nujol) 3200 (NH), 1717 (C=O), 1680 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 3, *J* = 6.6 Hz, $CHCH_3$), 1.43 (t, 3, *J* = 7.8 Hz, CH_2CH_3), 3.10 (s, d, $NHCH_3$), 3.22 (q, partially obscured 2, CH_2), 4.11 (m, 1, $CHCH_3$), 4.80 (d, 1, *J* = 3 Hz, CHS), 7.30–8.47 (m, 5, aromatic). Anal. ($C_{17}H_{19}N_3O_2S_2$) C, H, N, S.

Reaction of (±)-α-5-[1-(Indol-3-yl)ethyl]-2-methylamino-Δ²-thiazolin-4-one (1) with Cyclic Anhydrides. Sodium hydride (0.2 g, 0.008 mol) was added to a stirred solution of the thiazolinone **1** (1.08 g, 0.004 mol) in dry *N,N*-dimethylformamide (10 mL) under nitrogen. After 1 h, a solution of either succinic anhydride, glutaric anhydride, or phthalic anhydride (0.004 mol) in *N,N*-dimethylformamide (5 mL) was slowly added, and stirring was continued for 18 h. Water (20 mL) and saturated aqueous sodium bicarbonate (5 mL) were then added, and the basic solution was washed with ethyl acetate (3 × 25 mL) and acidified to pH 2 with 5 N hydrochloric acid. The oil which separated was dissolved in ethyl acetate (25 mL), and the residual aqueous solution was extracted with ethyl acetate (2 × 25 mL). The acidic ethyl acetate solutions were combined, dried (Na_2SO_4), and evaporated, yielding the crude carboxylic acids **8**, **10**, and **11**.

(±)-α-5-[1-(3-Carboxypropionyl)indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (8): 0.71 g, 48%; mp 208–210 °C (from methanol); IR ν_{max} (Nujol) 3200 (NH), 1743 (C=O), 1708 (C=O), 1680 (C=O), 1590 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (d, 3, *J* = 7 Hz, $CHCH_3$), 2.68 (t, 2, *J* = 8 Hz, CH_2), 2.95 (s, 0.9) and 3.02 (d, 2.1, *J* = 5 Hz) ($NHCH_3$), 3.33 (t, 2, *J* = 8 Hz, CH_2), 3.98 (m, 1, $CHCH_3$), 4.88 (d, 1, *J* = 3 Hz, CHS), 7.30–8.60 (m, 5, aromatic), 9.26 (q, 1, *J* = 5 Hz, $NHCH_3$). Anal. ($C_{18}H_{19}N_3O_4S$) C, H, N.

(±)-α-5-[1-[1-(4-Carboxybutyryl)indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (10): 0.58 g, 36%; mp 193–195 °C (from dichloromethane); IR ν_{max} (Nujol) 3200 (NH), 1725 (C=O), 1680 (C=O), 1595 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.29 (d, 3, *J* = 7 Hz, $CHCH_3$), 1.95 (m, 2, $CH_2CH_2CH_2$), 2.43 (t, 2, *J* = 6 Hz, CH_2CO), 2.92 (s, 0.9) and 3.00 (d, 2.1 *J* = 5 Hz) ($NHCH_3$), 2.85–3.25 (m, partially obscured, 2, CH_2CO), 3.91 (m, 1, $CHCH_3$), 4.88 (d, 1, *J* = 3.6 Hz, CHS), 7.20–8.60 (m, 5, aromatic), 9.28 (q, 1, *J* = 5 Hz, $NHCH_3$). Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N.

(±)-α-5-[1-[1-(2-Carboxybenzoyl)indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (11): 0.67 g, 40%; mp 204–206 °C (from ethyl acetate); IR ν_{max} (Nujol) 3150 (NH), 1740 (C=O), 1716 (C=O), 1690 (C=O), 1605 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.33 (d, 3, *J* = 7 Hz, $CHCH_3$), 2.76 (s, 0.8) and 2.84 (d, 2.2, *J* = 3 Hz) ($NHCH_3$), 3.79 (m, 1, $CHCH_3$), 4.75 (d, 1, *J* = 4.4 Hz, CHS), 6.90–8.40 (m, 9, aromatic), 9.00 (br s, 1, $NHCH_3$). Anal. ($C_{22}H_{19}N_3O_4O$) C, H, N.

2-Oxooxazolidinium Chlorides (12-15).[4] A solution of the appropriately substituted 2-dialkylaminoalkanol (0.01 mol) and triethylamine (0.01 mol) in dichloromethane (30 mL) was added dropwise to a stirred solution of 12.5% phosgene in toluene (25 mL) and dichloromethane (40 mL) at −40 °C under nitrogen. The cooling bath was then removed and after 90 min a white precipitate of the 2-oxooxazolidinium chloride (**12**–**15**) was collected, washed with dichloromethane, and dried.

3,3,4,4-Tetramethyl-2-oxooxazolidinium chloride (12): 48% yield; mp 93–94 °C; IR ν_{max} (Nujol) 1850 cm^{-1} (C=O).

3,3,5,5-Tetramethyl-2-oxooxazolidinium chloride (13): 61% yield; mp 94–94.5 °C; IR ν_{max} (Nujol) 1850 cm^{-1} (C=O).

4,4-Dimethyl-3-morpholino-2-oxooxazolidinium chloride (14): 25% yield; mp 95–96 °C; IR ν_{max} (Nujol) 1850 cm^{-1} (C=O).

3,3,5-Trimethyl-2-oxooxazolidinium chloride (15): 72% yield; mp 96–97 °C; IR ν_{max} (Nujol) 1850 cm^{-1} (C=O).

Basic Carbamic Esters. Sodium hydride (0.22 g, 0.009 mol) was added to a stirred solution of the thiazolinone **1** (1.08 g, 0.004 mol) in *N,N*-dimethylformamide (10 mL) under nitrogen. After 1 h, the mixture was cooled to 0 °C and the 2-oxooxazolidinium salt (**12**–**15**, 0.005 mol) added. After a further 1 h, ethyl acetate (25 mL) was added, the mixture was washed with water (3 × 10 mL) and dried (Na_2SO_4), and the solvent was evaporated. The crude product was chromatographed on silica gel using ethyl acetate–methanol (4:1) as the eluting solvent. Fractions containing the carbamic ester were collected and evaporated, and the solid obtained was recrystallized, yielding **(±)-α-5-[1-[1-[[(2-Dimethylamino-2-methylpropyl)oxy]carbonyl]indol-3-yl]-ethyl]-2-methylamino-Δ²-thiazolin-4-one (16):** 0.89 g, 54%; mp 130.5–131 °C (from acetone); IR ν_{max} (Nujol) 3300 (NH), 1745 (C=O), 1690 (C=O), 1590 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.0–1.4 [m, 9, $C(CH_3)_2$, $CHCH_3$], 2.29 [s, 6, $N(CH_3)_2$], 2.93 (s, 0.7) and 3.01 (s, 2.3) ($NHCH_3$), 3.90 (m, 1, $CHCH_3$), 4.38 (s, 2, CH_2), 4.88 (d, 1, *J* = 4 Hz, CHS), 7.2–8.4 (m, 5, aromatic), 9.37 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{21}H_{28}N_4O_3S$) C, H, N.

(±)-α-5-[1-[1-[(Dimethylamino-*tert*-butyloxy)carbonyl]indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (17): 0.21 g, 13%; mp 125–126 °C (from acetone–ether); IR ν_{max} (Nujol) 3200 (NH), 1740 (C=O), 1680 (C=O), 1580 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.21 (d, 3, *J* = 5 Hz, $CHCH_3$), 1.61 [s, 6, $C(CH_3)_2$], 2.31 [s, 6, $N(CH_3)_2$], 2.95 (s, 0.9) and 3.02 (d, 2.1, *J* = 5 Hz, collapses to singlet on D_2O exchange) ($NHCH_3$), 3.71 (s, 2, CH_2N), 3.86 (m, 1, $CHCH_3$), 4.81 (br s, 1, CHS), 7.2–8.3 (m, 5, aromatic), 9.21 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{21}H_{28}N_4O_3S$) C, H, N.

(±)-α-2-Methylamino-5-[1-[1-[[(2-morpholino-2-methylpropyl)oxy]carbonyl]indol-3-yl]ethyl]-Δ²-thiazolin-4-one (18): 0.33 g, 18%; mp 140–141 °C (from acetone); IR ν_{max} (KBr) 3240 (NH), 1730 (C=O), 1690 (C=O), 1590 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.12 [s, 6, $C(CH_3)_2$], 1.22 (d, 3, *J* = 6.8 Hz, $CHCH_3$), 2.50 [m, 4, $(CH_2)_2N$], 2.85 (s, 0.8) and 2.94 (s, 2.2) ($NHCH_3$), 3.53 [m, 4, $(CH_2)_2O$], 3.80 (m, 1, $CHCH_3$), 4.30 (s, 2, CH_2), 4.75 (d, 1, *J* = 4 Hz, CHS), 7.00–8.40 (m, 5, aromatic), 9.20 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{23}H_{30}N_4O_4S$) C, H, N.

(±)-α-5-[1-[1-(2-Dimethylamino-1-methylethoxycarbonyl)indol-3-yl]ethyl]-2-methylamino-Δ²-thiazolin-4-one (19): 0.41 g, 26%; mp 150–151 °C (from acetone); IR ν_{max} (KBr) 3240 (NH), 1730 (C=O), 1685 (C=O), 1590 cm^{-1} (C=N); ^{1}H NMR (Me_2SO-d_6) δ 1.21 (d, 3, *J* = 6.8 Hz, $CHCH_3$), 1.36 (d, 3, *J* = 6.8 Hz, $OCHCH_3$), 2.20 [s, 6, $N(CH_3)_2$], 2.48 [m, 2, $CH_2N(CH_3)_2$], 2.84 (s, 0.8) and 2.93 (s, 2.2) ($NHCH_3$), 3.80 (m, 1, $CHCH_3$), 4.75 (d, 1, *J* = 4 Hz, CHS), 5.18 (q, 1, *J* = 6.8 Hz, $OCHCH_3$), 6.80–8.30 (m, 5, aromatic), 9.10 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{20}H_{26}N_4O_3S$) C, H, N.

(±)-α-2-**Methylamino**-5-[1-[1-[[(2-**trimethylammonium**-2-**methylpropyl**)**oxy**]**carbonyl**]**indol**-3-**yl**]**ethyl**]-Δ^2-**thiazolin**-4-**one iodide** (**20**): Methyl iodide (0.4 mL) was added to a stirred solution of the *N*-dimethylamino-2-[[(methylpropyl)-oxy]carbonyl]thiazolinone **16** (0.30g) in dichloromethane (10 mL) at room temperature. After 18 h, the precipitated methiodide **20** was collected and recrystallized: 0.16 g, 40%; mp 182–183 °C (from methanol); IR ν_{max} (Nujol) 3200 (NH), 1750 (C=O), 1690 (C=O), 1592 cm^{-1} (C=N); ^1H NMR (Me_2SO-d_6) δ 1.24 (d, 3, J = 7 Hz, $CHCH_3$), 1.59 [s, 6, $C(CH_3)_2$], 2.92 (s, 0.9) and 2.96 (s, 2.1) ($NHCH_3$), 3.20 [s, 9, $N(CH_3)_3$], 3.89 (m, 1, $CHCH_3$), 4.74 (s, 2, CH_2), 4.89 (d, 1, J = 4 Hz, CHS), 7.24–8.44 (m, 5, aromatic), 9.34 (br s, 1, D_2O exchangeable, NH). Anal. ($C_{22}H_{31}IN_4O_3S$) C, H, N.

Antiviral Evaluation. (a) Tissue Culture Studies (Table I). Compounds were initially evaluated in simple diffusion assays. For this type of test, 10 mg/mL aqueous solutions of water-soluble compounds or 100 mg/mL solutions of insoluble compounds in dimethyl sulfoxide were diluted to a concentration of 1 mg/mL with Eagle's minimum essential medium, and 0.02 mL was introduced into wells 6 mm in diameter cut into an agar layer above virus-infected cell monolayers. Influenza A/Port Chalmers/1/73 (H3N2) strain was grown in MDCK cells, and Coxsackie B_1 virus was grown in HeLa cells. The diameters of zones around the wells free from virus-induced plaques (inhibition zones) are reported.

More accurate quantitation of antiviral activity was achieved by incorporation of varying concentrations of the test compound into the agar overlay above the virus-infected cell monolayers. The treated infected cells were incubated at 37 °C for 42 h in the Coxsackie assay and at 33 °C for 3 days in the influenza assay. The lowest concentration of each compound causing a ≥50% reduction in the number of plaques in comparison with untreated controls (PDD_{50}) is given. The reported minimum cytotoxic concentration (MCC) is the lowest concentration of compound causing morphological abnormalities in cell monolayers stained either with carbol fuchsin or neutral red.

(b) Influenza in Ferrets (Table II). (±)-α-5-[1-[1-[[(2-dimethylamino-2-methylpropyl)oxy]carbonyl]indol-3-yl]ethyl]-2-methylamino-Δ^2-thiazolin-4-one (**16**) hydrochloride was tested for antiviral activity in ferrets infected with influenza virus A/Port Chalmers/1/73 (H3N2). Nine ferrets (700–1500 g) were infected on day 0 by instilling 0.2 mL ($10^{4.0}$ EID_{50}) diluted virus intranasally without anaesthetic. One group of three ferrets remained untreated throughout the infection; a second group was treated with Ribavirin as a positive control and a third with **16**. Then, 0.2 mL doses of 50 mg/mL solutions of the compounds in distilled water were administered to unanaesthetized ferrets by intranasal instillation. Treatment commenced 2 h after infection and was repeated each hour to give a total number of seven doses on day 0 and nine doses on days +1, +2, and +3. Rectal temperatures were taken with a thermistor probe four times daily, both before and after infection, to establish base lines and the severity of the infection. Nasal washings were taken daily by the repeated introduction of small volumes of warmed saline into the anterior nares (total 20 mL) and collection of the expelled fluid in a Petri dish. Nasal washings were titrated in perspex trays and tested for the presence of hemagglutination by the addition of 0.5% washed chicken erythrocytes. Body weight was recorded throughout the experiment both as a measure of the severity of the infection and also as an indicator of compound toxicity. Blood samples were taken by cardiac puncture during Penthrane/N_2O/O_2 anaesthesia before and 10 days after infection and, after treatment with KIO_4 for removal of nonspecific inhibitors, were tested by hemagglutination inhibition for the presence of antibody to the infecting virus.

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References and Notes

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