

denoted by CMA and BAW, respectively. Unless otherwise stated, all reagents and solvents were reagent grade and used without subsequent purification.

Reaction of (+)- and (-)-Naltrexone Hydrochloride with *N*-Aminosuccinimide. To a stirred solution of (+)-naltrexone hydrochloride (110 mg, 0.29 mmol) and (-)-naltrexone hydrochloride (110 mg, 0.29 mmol) in DMF (2.5 mL) was added *N*-aminosuccinimide hydrochloride⁵ (200 mg, 1.33 mmol). The mixture was stirred at 100 °C for 18 h, and the solvent was removed in vacuo. Methanol, saturated sodium bicarbonate solution, and chloroform were added to the residue. The mixture was filtered (Celite), and the filtrate was extracted with chloroform (three times). The combined organic phases were washed with brine, dried, and concentrated to give a crude product that was purified on a Sephadex column (LH-20, MeOH) to afford pure meso isomer **2** (50 mg, 26%) and (±)-**1** (90 mg, 46.9%). Data for **2**: *R*_f, 0.25 (CMA 18:2:0.1); IR (KBr, cm⁻¹) 3400, 3000, 2830, 1640, 750; ¹H NMR (CDCl₃) δ 0.12 (4 H, m), 0.52 (4 H, m), 0.82 (2 H, m), 1.63 (2 H, d, *J* = 8.2 Hz), 2.00–2.50 (12 H, m), 2.65 (4 H, m), 3.06 (2 H, d, *J* = 18.4 Hz), 3.20 (2 H, d, *J* = 5.8 Hz), 5.56 (2 H, s), 6.45 (2 H, d, *J* = 7.8 Hz), 6.60 (2 H, d, *J* = 7.8 Hz); FABMS 662.2 (M⁺ + 1), 660.1 (M⁺ - 1). Data for **2**·2HCl: mp >265 °C dec; *R*_f, 0.37 (BAW, 2:1:1); [α]_D²⁵ -2.1° (c 0.1, MeOH). Anal. (C₄₀H₄₃O₆N₃·2HCl·4H₂O) C, H, N, Cl. Data for (±)-**1**: *R*_f, 0.36 (CMA, 18:2:0.1); IR (KBr, cm⁻¹) 3400, 2920, 2830, 1630, 1500, 1458; ¹H NMR (CDCl₃) δ 0.13 (4 H, m), 0.52 (4 H, m), 0.84 (2 H, m),

1.67 (2 H, d, *J* = 9.5 Hz), 2.15–2.50 (12 H, m), 2.65 (4 H, m), 3.05 (2 H, d, *J* = 18.4 Hz), 3.16 (2 H, d, *J* = 6.0 Hz), 5.50 (2 H, s), 6.46 (2 H, d, *J* = 8.0 Hz), 6.63 (2 H, d, *J* = 8.0 Hz); FABMS 662.2 (M⁺ + 1), 660.1 (M⁺ - 1). Data for (±)-**1**·2HCl: mp >265 °C dec; [α]_D²⁵ -5.2° (c 0.1, MeOH); *R*_f, 0.46 (BAW, 2:1:1). Anal. (C₄₀H₄₃O₆N₃·2HCl·4H₂O) C, H, N, Cl.

(5*S*,5'*S*,9*S*,9'*S*,13*R*,13'*R*,14*R*,14'*R*)-17,17'-Bis(cyclopropylmethyl)-6,6',7,7'-tetrahydro-4,5:4',5'-diepoxy-6,6'-(imino)[7,7'-bimorphinan]-3,3',14,14'-tetrol, (+)-**1**. The procedure was identical with that employed for the preparation² of (-)-**1** except that (+)-naltrexone hydrochloride (50 mg, 0.13 mmol) was employed in place of (-)-naltrexone hydrochloride. Column chromatography (Sephadex L-20, MeOH) afforded pure (+)-**1** (20 mg, 40%): *R*_f, 0.36 for free base (CMA, 18:2:0.1) and 0.46 for hydrochloride (BAW, 2:1:1); IR of (+)-**1** was identical with that of nor-BNI, (-)-**1**.

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Registry No. (-)-**1**, 105618-26-6; (+)-**1**, 114375-46-1; (±)-**1**, 114375-45-0; **2**, 114375-44-9; (+)-naltrexone-HCl, 114274-32-7; (-)-naltrexone-HCl, 16676-29-2; *N*-aminosuccinimide, 19283-13-7.

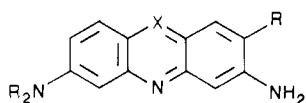
7-Aminoquinolines. A Novel Class of Agents Active against Herpesviruses

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A series of 7-aminoquinoline derivatives was synthesized and evaluated for their capacity to produce cytotoxicity in KB cells and to inhibit the replication of herpes simplex virus (HSV) type 1. All compounds tested inhibited the replication of HSV-1 with 50% inhibitory concentrations in the range of 2–50 μg/mL. The antiviral activity of many compounds, however, was separated from cytotoxicity to replicating uninfected cells by only two- to fivefold higher than those required for antiviral activity. Nonetheless, six compounds (**10**, **28**, **29**, **32**, **34**, and **36**) were identified in which the separation was greater than fivefold. All compounds examined were more potent inhibitors of viral DNA synthesis than the cellular DNA synthesis.

In recent years considerable research efforts have been conducted in order to find drugs useful for the treatment of herpes virus infections.^{1–5} Also, herpes viruses have been implicated as the cause of a number of carcinomas.^{6–9} Heterocyclic dyes, such as neutral red (**1a**) and proflavine (**1b**), are among the wide variety of compounds that exhibit



1a: R = Me; X = N
1b: R = H; X = CH

activity against herpesviruses.^{1,10,11} These drugs act by intercalation and photodynamic disruption of DNA. Although these dyes are not appropriate for human therapy,¹¹ another group of planar heterocyclic compounds—the 4-aminoquinolines such as chloroquine—act through intercalation¹² and have extensive therapeutic usefulness.¹³ Because 7-aminoquinolines may be considered analogues of proflavine and because they are relatives of chloroquine,

we have prepared and tested a series of such compounds. This article describes the synthesis and evaluation of novel

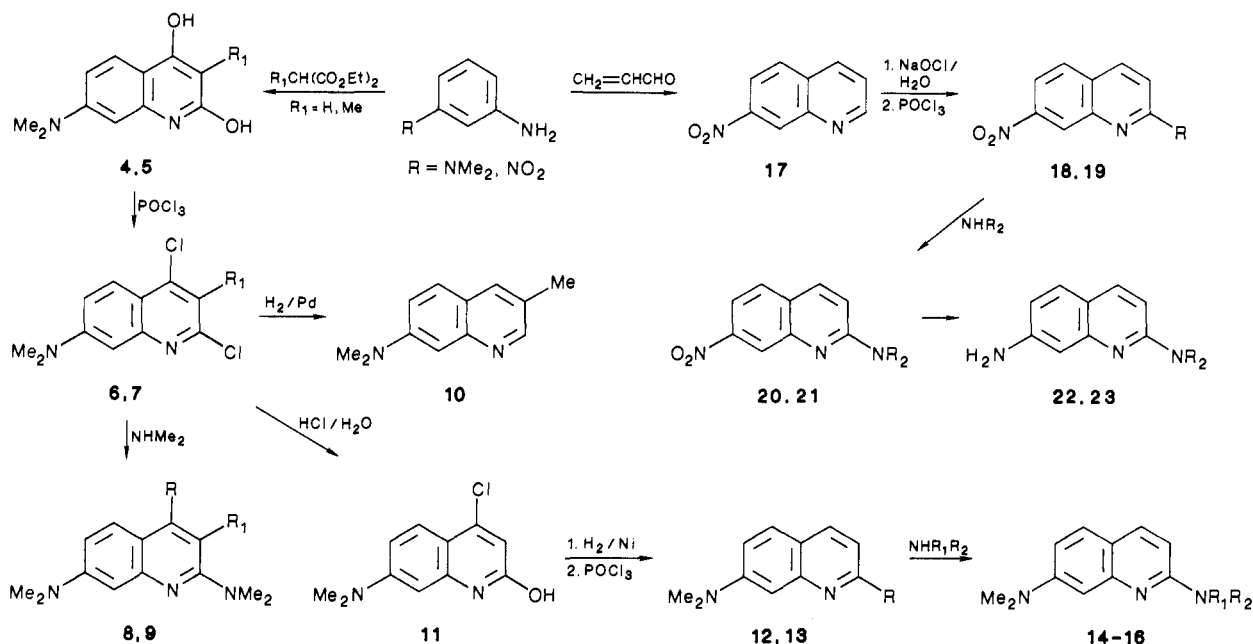
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Scheme I

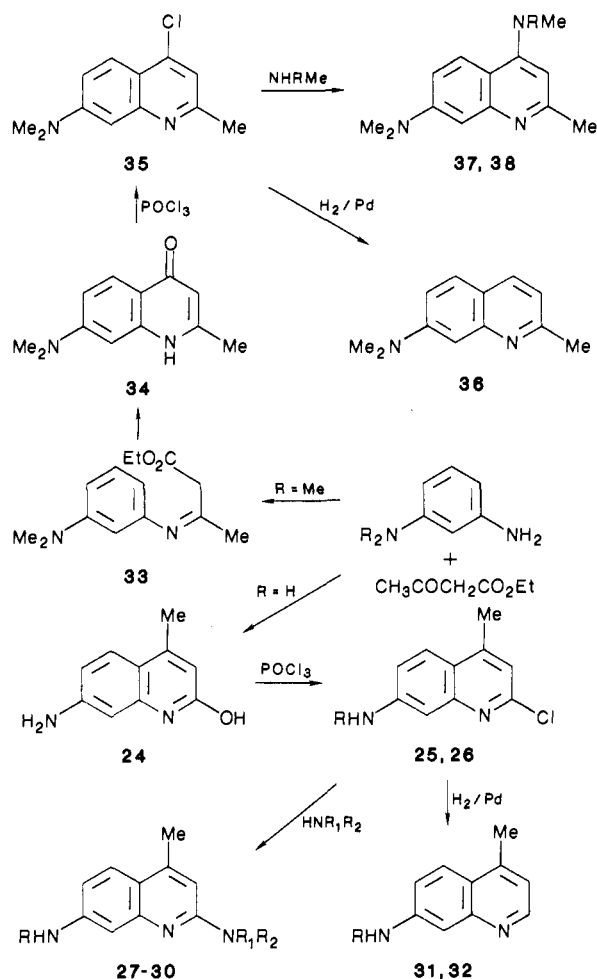


2,7- and 4,7-quinolinediamine and 7-quinolinamine derivatives as potential antiherpes agents.

Chemistry. Methods for the synthesis of the aminoquinolines are depicted in Schemes I and II. In scheme I, 2,4-dihydroxy-7-(dimethylamino)quinoline (4)¹⁴ and the 3-methyl derivative 5 were prepared, respectively, through condensation of *N,N*-dimethyl-*m*-phenylenediamine with diethyl malonate and diethyl methylmalonate. Reaction of 4 and 5 with POCl_3 gave the corresponding 2,4-dichloro derivatives 6 and 7. Condensation of these 2,4-dichloro derivatives 6 and 7 with dimethylamine gave the corresponding substituted amines 8 and 9. Although condensation of 6 with dimethylamine gave the 2,4-disubstituted amine 8, only the 2-amino substituent 9 was obtained through the condensation of 7 with dimethylamine. This can be explained because the chlorine at position 2 of quinoline is more reactive to nucleophilic substitution than at position 4.¹⁵ Catalytic hydrogenolysis of 7 gave 3-methyl-7-(dimethylamino)quinoline (10). Hydrolysis of 2,4-dichloroquinoline (6) gave the corresponding 4-chlorocarbostyryl 11. Catalytic hydrogenolysis of 4-chloro-7-(dimethylamino)carbostyryl (11) gave 7-(dimethylamino)carbostyryl (12), which upon treatment with POCl_3 gave the 2-chloro derivative 13. Condensation of 13 with ammonia, methylamine, and dimethylamine afforded the corresponding 2,7-quinolinediamines 14–16.

With 7-nitroquinoline (17) as the starting material, prepared by the Skraup reaction with *m*-nitroaniline,¹⁶ the required 2,7-quinolinediamine (22) and *N,N*-dimethyl-2,7-quinolinediamine (23) were synthesized as described in Scheme I. Treatment of 17 with sodium hypochlorite and water gave 7-nitrocarbostyryl (18),¹⁷ which was treated with POCl_3 , and the resultant 2-chloro derivative 19 was condensed with ammonia in a bomb under pressure to give 20, which upon catalytic hydrogenation gave 22. By the same method 19 was condensed with dimethylamine and

Scheme II



the resultant 21 was reduced with SnCl_2/HCl to 23.

The synthesis of 4-methyl-2,7-quinolinediamines 27–30 (Table I) is described in Scheme II. The condensation of *m*-phenylenediamine with ethyl acetoacetate gave 7-amino-4-methylcarbostyryl (24).^{18,19} Chlorination of 24

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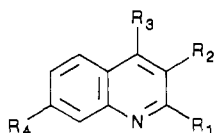
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Table I. Physical Properties and Antiviral Activity of 7-Aminoquinolines



compd no.	R ₁	R ₂	R ₃	R ₄	recrystn solvent	mp, °C	method	yield, %	formula	IC ₅₀ , µg/mL ^a		
										cytotoxicity	HSV-1 replication	in vitro therapeutic index ^b
5	OH	Me	OH	NMe ₂	ethanol	352-355	A	75	C ₁₂ H ₁₄ N ₂ O ₂	80	29	3
6	Cl	H	Cl	NMe ₂	hexane	108-110	B	72	C ₁₁ H ₁₀ Cl ₂ N ₂	c		
7	Cl	Me	Cl	NMe ₂	hexane	117-119	B	77	C ₁₂ H ₁₂ Cl ₂ N ₂	>32 ^d	13	2
8	NMe ₂	H	NMe ₂	NMe ₂	hexane	118-120	C	99	C ₁₅ H ₂₂ N ₄	19	5	4
9	NMe ₂	Me	Cl	NMe ₂	ethyl acetate	135-137	C	89	C ₁₄ H ₁₆ ClN ₃	>32 ^d	49	
10	H	Me	H	NMe ₂	petr ether	54-56	D	48	C ₁₂ H ₁₄ N ₂	70	7.6 ^e	9
11	OH	H	Cl	NMe ₂	ethanol	279-283	E	99	C ₁₁ H ₁₁ ClN ₂ O			
12	OH	H	H	NMe ₂	ethanol	240-242	D	90	C ₁₁ H ₁₂ N ₂ O	139	30	5
13	Cl	H	H	NMe ₂	hexane	105-107	B	94	C ₁₁ H ₁₁ ClN ₂	c		
14	NH ₂	H	H	NMe ₂	hexane	140-142	F	30	C ₁₁ H ₁₃ N ₃	10	2 ^e	5
15	NHMe	H	H	NMe ₂	hexane	115-117	C	71	C ₁₂ H ₁₆ N ₃	24	11	2
16	NMe ₂	H	H	NMe ₂	H ₂ O/methanol	106-108	C	86	C ₁₃ H ₁₇ N ₃	17	6 ^e	3
19	Cl	H	H	NO ₂	ethanol	137-139	B	95	C ₉ H ₅ ClN ₂ O ₂			
20	NH ₂	H	H	NO ₂	acetone	247-249	C	90	C ₉ H ₇ N ₃ O ₂			
21	NMe ₂	H	H	NO ₂	methanol	168-170	C	79	C ₁₁ H ₁₁ N ₃ O ₂	c		
22	NH ₂	H	H	NH ₂	ether	147-149	D	96	C ₉ H ₉ N ₃	40	10	4
23	NMe ₂	H	H	NH ₂	ether	105-106	f	50	C ₁₁ H ₁₃ N ₃	130	51	3
25	Cl	H	Me	NH ₂	H ₂ O/methanol	126-128	B	78	C ₁₀ H ₉ ClN ₂	133	53	3
26	Cl	H	Me	NHAc	acetone	205-207	f	99	C ₁₂ H ₁₁ ClN ₂ O	86 ^e	21 ^e	4
27	NH ₂	H	Me	NHAc	chloroform	237-239	F	25	C ₁₂ H ₁₃ N ₃ O	34	10 ^e	3
28	NMe ₂	H	Me	NHAc	acetone	229-231	C	74	C ₁₄ H ₁₇ N ₃ O	>100 ^d	18	6
29	NH ₂	H	Me	NH ₂	ethanol	325-327	f	82	C ₁₀ H ₁₁ N ₃ ·HCl	27	4 ^e	7
30	NMe ₂	H	Me	NH ₂	methanol/H ₂ O	147-149	C	70	C ₁₂ H ₁₅ N ₃	11 ^e	6 ^e	2
31	H	H	Me	NH ₂	hexane	79-81	D	25	C ₁₀ H ₁₀ N ₂	63	40 ^{d,e}	2
32	H	H	Me	NHAc	benzene	201-202	D	75	C ₁₂ H ₁₂ N ₂ O	215 ^e	31 ^e	7
34	Me	H	OH	NMe ₂	ethanol	325-328	f	30	C ₁₂ H ₁₄ N ₂ O	447 ^e	23 ^e	19
35	Me	H	Cl	NMe ₂	hexane	90-92	B	76	C ₁₂ H ₁₃ ClN ₂	22	7	3
36	Me	H	H	NMe ₂	hexane	59-62	E	84	C ₁₂ H ₁₄ N ₂	40	4.5 ^e	9
37	Me	H	NMe ₂	NMe ₂	ethyl acetate	78-80	C	60	C ₁₄ H ₁₉ N ₃	15	9	2
38	Me	H	NHMe	NMe ₂	ethanol	266-270	C	25	C ₁₃ H ₁₇ N ₃	14	6	2

^a 50% inhibitory concentration, see text for methods. ^b Ratio of cytotoxicity to inhibition of viral replication, rounded to nearest whole numbers. ^c Compound insufficiently soluble to be tested in vitro. ^d Low drug solubility, IC₅₀ not reached at maximum solubility. ^e Average of replicate analyses performed at two or three separate times. ^f Method described in the Experimental Section. All compounds had elemental analysis for C, H, N.

with POCl₃ gave the 2-chloro derivative **25**. Acetylation of **25** gave the 7-acetamido derivative **26**, which was aminated to **27** and **28**. Hydrolysis of **27** gave 4-methyl-2,7-quinolinediamine (**29**). Compound **25** was directly condensed with dimethylamine to give **30**. A similar condensation failed with ammonia or methylamine. Catalytic hydrogenation of **25** and **26** gave 7-amino- and 7-acetamido-4-methylquinolines (**31** and **32**).

The synthesis of 2-methyl-4,7-quinolinediamines **37** and **38** is also described in Scheme II. The condensation of *N,N*-dimethyl-*m*-phenylenediamine with ethyl acetate in refluxing benzene gave the intermediate β -*m*-(dimethylamino)anilino crotonic ester (**33**). Heating **33** at 260 °C in phenyl ether gave 7-(dimethylamino)-2-methyl-4(1*H*)-quinolone (**34**). Chlorination of **34** gave the 4-chloro derivative **35**, which upon condensation with dimethylamine and methylamine, gave the 4-amino derivatives **37** and **38**. Catalytic hydrogenolysis of **35** gave 7-(dimethylamino)-2-methylquinoline (**36**).

Antiviral Activity. The quinolines were evaluated for antiviral activity by comparing effects on titers of herpes simplex virus type 1 (HSV-1) in infected cells to effects

Table II. Antiviral Activity of Known Drugs^a

compd	IC ₅₀ , µg/mL		
	cytotoxicity	HSV-1 replication	in vitro therapeutic index
acyclovir ^b	>100	2.6	>38 ^d
ara-A	39	5.6	7
ara-T	>500 ^c	0.6	833
phosphonoformate	1490	189	8
arildone	75	11	7
chloroquine	85	27	3

^a All terms and units defined in legend to Table I. ^b Averages of replicate experiments. ^c I₅₀ concentrations not reached at highest level tested (500 µg/mL). ^d Plaque reduction assay with HSV-1 strain 148.

on four biochemical parameters in uninfected cells. The latter effects on protein and DNA synthesis were taken as measures of cytotoxicity. The ratio between cytotoxicity and inhibition of virus replication was called the "in vitro therapeutic index". Table I summarizes results with compounds for which adequate amounts were available and which were sufficiently soluble in cell culture medium to be evaluated. All 24 compounds tested inhibited the replication of HSV-1; 50% inhibitory (I₅₀) concentrations ranged from 2 to 50 µg/mL. All compounds also inhibited cellular protein and DNA synthesis but at generally higher concentrations (I₅₀ = 10-447 µg/mL). Thus, as a group,

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the compounds possessed a positive in vitro therapeutic index. Most compounds, however, had in vitro therapeutic indexes of only 2–4.

Several known antiherpes drugs that act by various mechanisms were evaluated for comparative purposes (Table II). Acyclovir is highly specific because it is phosphorylated by herpes virus thymidine kinase but not by cellular kinases and therefore has a high therapeutic index. Vidarabine (9- β -D-arabinofuranosyladenine, *ara-A*) and phosphonoformate—two clinically useful antivirals that act by selective inhibition of herpes DNA polymerase—differed markedly in their potency but had essentially the same in vitro therapeutic indexes. Arildone (4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3,5-heptanedione), an experimental antiviral drug, also had a similar in vitro therapeutic index. *ara-T* (1- β -D-arabino-furanosylthymine) was more potent and much more selective than the other compounds owing to its known preferential activation in herpes-infected cells by HSV-induced pyrimidine deoxynucleoside kinase.⁴ Chloroquine [7-chloro-4-[[4'-(diethylamino)-1'-methylbutyl]amino]quinoline], which is known to possess activity against herpesviruses in vitro^{21,22} but not in vivo,²² was more potent than phosphonoformate but was not as selective as the known antiviral drugs against HSV-1 replication. These observations, plus the known selectivity of other antivirals,¹⁻⁵ led us to conclude that unless in vitro antiviral activity is separated from cytotoxicity by at least seven- to eightfold, compounds probably do not merit additional consideration. On this basis, compounds, **10**, **29**, **32**, **34**, and **36** were considered to be active antivirals and merit additional investigation.

Experimental Section

Melting points were determined in a Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer spectrophotometer Model 337, in KBr disks on Nujol mulls. NMR spectra were run on a Varian A-60 spectrometer. The IR and NMR spectra were consistent with the assigned structures. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN. Where analyses are indicated only by the symbols of elements, they are within $\pm 0.4\%$ of the theoretical values. Hydrogenations were carried out in a Parr hydrogenator. The general procedures listed in Table I and the preparation of the requisite starting materials are described by the following examples.

Method A. 7-(Dimethylamino)-4-hydroxy-3-methylcarbostyryl (5). A mixture of 13.6 g (0.1 mol) of *N,N*-dimethyl-*m*-phenylenediamine and 17.4 g (0.1 mol) of diethyl methylmalonate was heated in an oil bath at 180–200 °C for 1.5 h with an alcohol separator. Only 4.5 g of ethanol was separated. The reaction mixture was then heated on a heating mantle at 260–270 °C. A yellow precipitate was obtained, and an additional 4.5 g ethanol was separated after 20 min of heating. The resulting yellow solid was cooled and washed with acetone, giving 15 g (75%) of **5**. Recrystallization from ethanol gave 15 g (75%) of **5**: ¹H NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.47 (s, 3 H, Me), 2.95 (s, 6 H, NMe₂), 6.47 (s, H-8), 6.72 (d, J = 9 Hz, H-6), 7.57 (d, H-5). Anal. (C₁₂H₁₄N₂O₂) C, H, N.

Method B. Chlorination of the 2- or 4-hydroxyquinolines was accomplished by the following general method. A mixture of 0.1 mol of the hydroxyquinoline and excess phosphorus oxychloride (0.2 mol) was heated gently until complete solution was achieved. The reaction mixture was refluxed for 1 h and then kept in ice to cool. It was diluted with water while being cooled. After dilution it was neutralized with NH₄OH, and the resulting pre-

cipitate was recrystallized from the proper solvent.

Method C. Condensation of the Chloroquinolines with Dimethyl- and Methylamine or Ammonia. A mixture of the chloroquinoline and excess amine or ammonia (15 equiv) in methanol was heated in a Parr pressure reaction bomb at 110–120 °C for 15 to 20 h. Usually the desired product precipitated after evaporation of the excess amine at room temperature.

Method D. Catalytic Hydrogenolysis of Chloroquinolines. 7-(Dimethylamino)carbostyryl (12). 4-chloro-7-(dimethylamino)carbostyryl (**11**) (2.2 g, 0.01 mol) was dissolved in 25 mL of H₂O containing 5 g of KOH, with addition of 5 mL of ethanol to make a clear solution. To the solution was added 0.5 g of Raney nickel, and hydrogen was allowed to pass through the solution with shaking. After 3 h the reaction mixture was filtered from the catalyst. Neutralization with HCl gave a crystalline product. Recrystallization from acetone gave 1.7 g of **12**: ¹H NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.97 (s, 6 H, NMe₂), 6.61 (s, H-8), 6.71 (d, H-6), 6.82 (d, H-4), 7.46 (d, H-5), 7.77 (d, H-3). Anal. (C₁₁H₁₂N₂O) C, H, N. Hydrogenolysis of the rest of the chloro derivatives was done with 5% Pd/C in glacial acetic acid solution. 2,7-Quinolinediamine (**22**) was prepared by hydrogenation of **20** by use of activated Raney nickel as catalyst in acetone solution.

Method E. 4-Chloro-7-(dimethylamino)carbostyryl (11). A mixture of 2.4 g (0.01 mol) of 2,4-dichloro-7-(dimethylamino)quinoline (**6**) and 15 mL of 6 N HCl was refluxed for 1 h. During reflux a red color, which developed at the start of the reaction, disappeared by the end of the reaction. The reaction mixture was diluted with water and neutralized with NaOH solution, whereupon a yellow crystalline product was obtained. Recrystallization from alcohol gave 2.2 g of **11**: ¹H NMR ($\text{Me}_2\text{SO}-d_6$) δ 3.01 (s, 6 H, NMe₂), 6.90 (s, H-8), 7.18 (s, H-3), 7.25 (d, H-6), 7.84 (d, H-5). Anal. (C₁₁H₁₁ClN₂O) C, H, N.

N²,N²-Dimethyl-2,7-quinolinediamine (23). A mixture of 0.2 g (0.001 mol) of **21** and 1.2 g of SnCl₂ in 5 mL of concentrated HCl was heated with stirring at 80 °C for 12 h. The reaction mixture was diluted with water and neutralized with NaOH solution. The product obtained was recrystallized from ether, giving 0.1 g of **23**: ¹H NMR (CDCl₃) δ 2.97 (s, 6 H, NMe₂), 5.32 (s, NH₂), 6.61 (s, H-8), 6.71 (d, H-6), 6.82 (d, H-4), 7.46 (d, H-5), 7.77 (d, H-3). Anal. (C₁₁H₁₃N₃) C, H, N.

Method F. N⁷,N⁷-Dimethyl-2,7-quinolinediamine (14). A mixture of 2.6 g (0.01 mol) of 2-chloro-7-(dimethylamino)quinoline (**13**) and 15 g of phenol was allowed to heat at 140 °C. Dry ammonia was passed through the solution at 180–200 °C for 6 h. The mixture was treated with a strong NaOH solution and then extracted with chloroform. The chloroform was evaporated, and the product obtained was recrystallized from hexane to give 0.62 g of **14**: ¹H NMR (CDCl₃) δ 3.09 (s, 6 H, NMe₂), 6.61 (s, H-8), 6.71 (d, H-6), 6.81 (d, H-4), 7.42 (d, H-5), 7.69 (d, H-3). Anal. (C₁₁H₁₃N₃) C, H, N.

4-Methyl-2,7-quinolinediamine Hydrochloride (29). A mixture of 0.4 g (0.002 mol) of **27** and 10 mL of 15% HCl was refluxed for 1 h. The reaction mixture was neutralized with NH₄OH solution after which most of the product stayed in solution. The solution was filtered and cooled, whereupon a yellow crystalline material was obtained. Recrystallization from ethanol gave 0.3 g of **29**. Anal. (C₁₀H₁₁N₃HCl) C, H, N.

7-(Dimethylamino)-2-methyl-4(1H)-quinolone (34). A mixture of 13.6 g (0.1 mol) of *N,N*-dimethyl-*m*-phenylenediamine and 13 g (0.1 mol) of ethyl acetoacetate in 200 mL of benzene was refluxed for 7 h with a water separator. The benzene was distilled, and the oily residue obtained was heated at 255 °C in phenyl ether for 0.5 h. The solid obtained was then separated after cooling and washed with petroleum ether. Recrystallization from ethanol gave 6.5 g of **34**: ¹H NMR ($\text{Me}_2\text{SO}-d_6$) δ 2.39 (s, Me), 2.98 (s, 6 H, NMe₂), 6.11 (s, H-3), 6.72 (s, H-8), 6.95 (d, H-6), 7.92 (d, H-5). Anal. (C₁₂H₁₄N₂O) C, H, N.

Virus and Cells. The source of cells and HSV-1 (HF strain), the routine growth and passage of KB and BHK 21/4 cells, the propagation and titration of virus, and techniques for the enumeration of cells and detection of mycoplasma contamination have been described previously.²⁴

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Inhibition of HSV-1 Replication. The effect of drugs on virus replication was measured as described previously²⁵ by treating duplicate cultures of HSV-1-infected KB cells for 20 h with one of five or six half-log dilutions of test compound. Virus titers were determined by enumeration of macroscopic plaques in BHK 21/4 cells seeded with serial dilutions of the disrupted KB cell cultures.

Cytotoxicity Determinations. Biochemical cytotoxicity measurements were made as detailed earlier²⁶ with logarithmically growing KB cells planted in scintillation vials. Two sets of duplicate cultures were treated for 20 h with one of five or six half-log dilutions of drug. One set of cultures was used to determine drug effects on protein synthesis as measured both by Lowry assays and incorporation of ³H-labeled amino acids into acid-precipitable material. The other was used to determine drug effects on DNA synthesis as measured by diphenylamine assay and incorporation of [³H]thymidine.

Data Analysis. Dose-response relationships were constructed by linearly regressing log drug concentrations against the percent inhibition values derived for viral replication, incorporation of

[³H]thymidine, incorporation of ³H-labeled amino acids, and net synthesis of total protein or total DNA. *I*₅₀ concentrations were calculated from the regression lines by using methods previously described.²⁵ Samples containing 10 μg/mL *ara*-A were included in all assays as positive controls. Results from sets of assays were rejected whenever inhibition by *ara*-A deviated from its mean response by more than 1.5 standard deviations. *I*₅₀ concentrations for cytotoxicity presented in the tables are the mean values of the four *I*₅₀ concentrations for effects on protein and DNA synthesis.

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Registry No. 4, 31136-93-3; 5, 114058-60-5; 6, 114058-61-6; 7, 114058-62-7; 8, 114058-63-8; 9, 114058-64-9; 10, 114058-65-0; 11, 114058-66-1; 12, 101340-84-5; 13, 114058-67-2; 14, 114058-68-3; 15, 114058-69-4; 16, 114058-70-7; 17, 613-51-4; 18, 75755-37-2; 19, 49609-03-2; 20, 49609-04-3; 21, 114058-71-8; 22, 114058-72-9; 23, 114058-73-0; 24, 19840-99-4; 25, 114058-74-1; 26, 52507-64-9; 27, 114058-75-2; 28, 114058-76-3; 29-HCl, 114058-77-4; 30, 114058-78-5; 31, 114058-79-6; 32, 114058-80-9; 33, 114058-81-0; 34, 114058-82-1; 35, 114058-83-2; 36, 114058-84-3; 37, 114058-85-4; 38, 114058-86-5; *N,N*-dimethyl-*m*-phenylenediamine, 2836-04-6; dimethyl malonate, 105-53-3; ethyl acetoacetate, 141-97-9.

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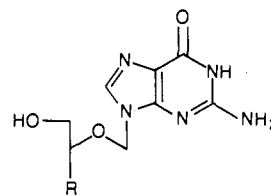
Synthesis and Antiviral Activity of Novel N-Substituted Derivatives of Acyclovir[†]

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Novel N-substituted derivatives of acyclovir (**1a**) were synthesized and evaluated for their antiviral, antimetabolic, and antitumor cell properties in vitro. Monomethylation of **1a** at positions 1, 7, and N-2 gave compounds **2-4**, respectively. When positions 1 and N-2 were linked together by an isopropeno group, the tricyclic 9-[(2-hydroxyethoxy)methyl]-1,*N*-2-isopropenoguanine (**5**) was obtained. Compound **5** was then further methylated at positions N-2 and 7 to give **6** and **7**, respectively. None of the new acyclovir derivatives showed any appreciable antimetabolic or antitumor cell activity. However, compounds **2** and **5** exhibited a marked antiherpetic activity. Their activity spectrum was similar to that of acyclovir, and their selectivity as inhibitors of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) was at least as great as, if not greater than, that of acyclovir.

The potent and selective antiherpetic activity of the acyclic analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, **1a**),^{1,2} has generated much interest in the synthesis of new congeners. Modifications of the acyclic side chain of **1a** have given rise to several new compounds with significant selective antiviral activity,³⁻¹⁴ 9-(1,3-dihydroxy-2-propoxymethyl)guanine (**1b**)³⁻⁸ being the acyclovir derivative that has been most extensively pursued for its antiviral properties¹⁵ (for recent reviews on acyclic guanosine analogues, see ref 16 and 17). Modifications of the guanine moiety of acyclovir have received relatively minor attention and yielded few compounds with appreciable antiviral activity. Only the 8-substituted derivatives, i.e. 8-amino-, 8-bromo-, 8-iodo-, and 8-methylacyclovir have proven to be active antiherpetic agents in vitro.¹⁸



1a. R = H
b. R = CH₂OH

Acyclovir owes its antiherpetic selectivity to a specific phosphorylation by the virus-encoded deoxythymidine

[†] Abbreviations used: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VV, vaccinia virus; VSV, vesicular stomatitis virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; SHV-1, suid herpesvirus type 1; BHV-1, bovid herpesvirus type 1; EHV-1, equid herpesvirus type 1; TK⁻, deficient in thymidine kinase inducing activity; PRK, primary rabbit kidney; HEL, human embryonic lung.

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