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Antiviral Research 42 (1999) 121–137

Antiviral Research

Synthesis and anti-influenza virus activity of novel pyrimidine derivatives

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Received 8 December 1997; accepted 6 February 1999

Abstract

Efficient synthetic routes of 2-amino-4-(ω-hydroxyalkylamino)pyrimidine derivatives were investigated in relation to the anti-influenza virus activity of these compounds. The derivatives in which cyclobutyl and cyclopentyl groups were introduced to the β-position of the aminoalkyl group (especially the cyclobutyl group substituted by a phenylalkyl group at the 3′-position) resulted in improved antiviral potency: i.e. an average 50% effective concentration for inhibition of plaque formation (EC_{50}, μM) of 0.1–0.01 μM for both types A and B influenza virus. The antiviral efficacies were in the order of amino group > hydroxyiminomethyl group > halogen substitution at the 5-position, and chlorine or methoxy group > hydrogen at the 6-position of the pyrimidine ring. The antiviral indices of these compounds were 2–6 with respect to the 50% inhibitory concentration for cell proliferation (IC_{50}, μM) for growing cells, but > 500 to $> 10^4$ with respect to the IC_{50} for stationary cells, indicating that these compounds may be efficacious for the topical treatment of influenza virus infection.

Keywords: Influenza viruses A and B; Herpes simplex virus types 1 and 2; Cytomegalovirus; Anti-influenza agents; Pyrimidine derivatives; Structure–antiviral activity relationships

1. Introduction

Viral lower respiratory diseases, especially influenza, constitute a serious health care problem throughout the world (Shaw et al., 1992; LaForce et al., 1994). Because of the antigenic drift and

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0166-3542/99/$ - see front matter
PII: S0166-3542(99)00019-4

antigenic shift of influenza viruses, influenza virus vaccines have low efficacy against influenza pandemics, and improved influenza virus vaccines are sought (Cox et al., 1994; Ghendon, 1994; Gravenstein et al., 1994; Oka et al., 1994; Tamura et al., 1995). New anti-influenza virus agents are also being tested and developed (Shigeta et al., 1992; Von Itzstein et al., 1993; Thomas et al., 1994; Tomassini et al., 1994; Jedrzejas et al., 1995; Nagai et al., 1995; Kurihara et al., 1996; Kim et al., 1997; Tuzikov et al., 1997). Influenza viruses contain their own specific RNA polymerase within the virus particles. This RNA polymerase is composed of three subunits, PB1, PB2 and PA. The binding of PB2 to the 5′-terminal cap of host mRNA is accompanied by endonucleolytic cleavage at a purine residue (usually adenine) 10–13 nucleotides away from the cap. PB1 then catalyzes the extension of the primer, using the viral (−)RNA strand as template, adding first a guanine residue, and then continuing the transcription of the whole template (Nakagawa et al., 1995, 1996). This elongation of the primer after an adenine residue occurs via the incorporation of a guanine residue that is complementary to the second nucleotide from the 3′ end of the viral RNA segments (Lamb and Choppin 1983; Toyoda et al., 1996). We have therefore investigated the anti-influenza virus activity of several guanosine analogues. During our search, some 2-amino-4-(ω-hydroxyalkylamino)pyrimidine derivatives (intermediate compounds for the synthesis of guanosine analogues from 2,4-diaminopyrimidine) showed anti-influenza virus activity. Although several reports on the synthesis of derivatives of 2,4-diaminopyrimidine have been presented (Mengel et al., 1984, 1988; Boumchita et al., 1990, 1991; Legraverend et al., 1990), no study of the antiviral activity of these compounds was reported.

In the present study, we investigated the efficient synthetic routes of 2-amino-4-(ω-hydroxyalkylamino)pyrimidine derivatives from 2,4-diamino-pyrimidine, and determined the relationship between the chemical structure and the in vitro anti-influenza virus activity, cell growth (proliferation) inhibition and cytotoxicity of these derivatives.

2. Materials and methods

2.1. Chemical synthesis of compounds

Compounds **1** to **3** (Table 1) were commercially available. The 2-amino-4-(ω-hydroxyalkylamino)-pyrimidine derivatives (compounds **4**–**17**, **19**–**30**, **32**, **40** and **41**) (Tables 1–5) were all prepared from the corresponding ω-hydroxyalkylamine derivatives (M. Hisaki, S. Imabori, F. Iwakura, M. Azuma, and T. Suzutani, Jpn. Patent 08-134044) by reaction with the corresponding 2-amino-4-chloro-5,6-substituted pyrimidine derivatives and triethylamine in ethanol. The 2,5-diamino-4(ω-hydroxyalkylamino)pyrimidine derivatives (compounds **33**–**37**) (Table 5) were prepared by the method of Shealy and Clayton (1973) from the corresponding compounds **8** and **24**–**26** by the diazo-coupling reaction and then reduction. Compound **39** (2-amino-5-hydroxyiminomethyl-6-chloro-4-[[[3-(2-phenylethyl)-1-hydroxymethyl-1-cyclobutyl]methyl]amino]pyrimidine) (Table 5) was prepared from the corresponding 2-amino-5-formyl-4-(ω-hydroxyalkylamino)pyrimidine derivatives and hydroxyamine hydrochloride by refluxing in ethanol. Compounds **18**, **42**, **43**, **45** and **46** (Tables 3 and 6) were prepared by reduction with palladium hydroxide from the corresponding 6-chloro-4-(ω-hydroxyalkylamino)-pyrimidine derivatives. The same conditions were used for the preparation of 2,5-diamino-4-(ω-hydroxyalkylamino)pyrimidines (compounds **47** and **48**) (Table 6) from the corresponding 2-amino-5-diazo-6-chloro-4-(ω-hydroxyalkylamino)pyrimidine derivatives. Compound **44** (Table 6) was prepared by the treatment of compound **43** with bromine and acetic acid. Compound **31** (Table 4) was prepared by the treatment of compound **32** with 20% HCl. Compound **50** (Table 6) was prepared from a 5-hydroxyiminomethylpyrimidine derivative (compound **45**) by treatment with acetic acid. The treatment of compound **50** with ammonia in methanol solution gave compound **49** (Table 6). Compound **38** (Table 5) was prepared from compound **40** by reduction. The nuclear magnetic resonance spectra of all compounds synthesized were consistent with their assigned

structures. All compounds gave satisfactory mass spectral results. The analytical data for these compounds, except for compounds **1** to **3** which are already known, are shown in Table 8. All compounds were dissolved in DMSO at 10 mg/ml just before the biological activity assays, and serially diluted in culture medium.

2.2. Cells and viruses

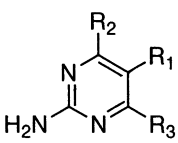
The MDCK cells were kindly provided by RIKEN Cell Bank (Tsukuba, Japan), and cultivated with Eagle's minimum essential medium (MEM) supplemented with 10% newborn calf serum (MEM-NCS10). Human embryo lung fibroblast (HEL) cells were established in our laboratory with MEM-NCS10, and used for the experiments within 20 passages (Suzutani et al., 1988).

The A/PR/8/34(H1N1) strain of influenza virus A, passaged in our laboratory, and the B/Gifu/2/73 strain of influenza virus B, kindly supplied by Dr H. Kida (Hokkaido University School of Veterinary Medicine, Sapporo, Japan), were propagated in 11-day-old embryonated eggs. The VR-3 strain of herpes simplex virus type 1, the UW-268 strain of herpes simplex virus type 2 and the AD-169 strain of human cytomegalovirus were generously supplied by the American Type Culture Collection (Rockville, MD) and grown in HEL cells. All viruses were titrated by a plaque forming assay and stored in small aliquots at −80°C.

2.3. Assay of antiviral activity of test compounds

The assay of the antiviral activities of the test compounds against influenza viruses was carried

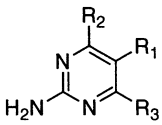
Table 1
Anti-influenza virus and anti-proliferative activity of pyrimidine derivatives

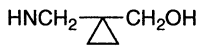
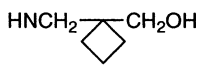
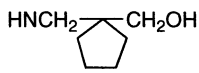
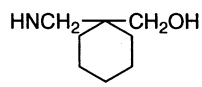
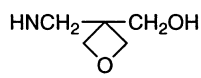
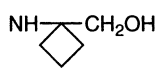
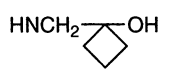
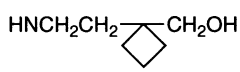
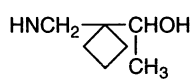
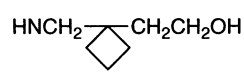
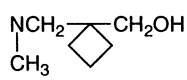


No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for growing MDCK cells
				Type A	Type B	
1[b]	H	H	NH_2	>910	>910	>910
2[b]	H	Cl	NH_2	>690	>690	>690
3[b]	NH_2	Cl	NH_2	>630	>630	ND[a]
4	H	Cl	$NHCH_2CH_2CH_2OH$	>490	>490	>490
5	H	Cl	$NHCH_2C(H_3C)(CH_3)CH_2OH$	250	220	ND
6	H	Cl	$NHCH_2C(H_5C_2)(C_2H_5)CH_2OH$	190	120	220

[a] ND, Not done.
[b] Known compound.

Table 2
Anti-influenza virus, anti-proliferative and cytotoxic activity of pyrimidine derivatives



No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for MDCK cells	
				Type A	Type B	Growing	Stationary
7	H	Cl		>440	>440	>440	>440
8	H	Cl		82–140	45–58	33–58	>410
9	H	Cl		26–51	22–32	5.5–39	>390
10	H	Cl		110	150	ND[a]	ND
11	H	Cl		>410	>410	>410	>410
12	H	Cl		>440	220	>440	>440
13	H	Cl		>130	>130	ND	ND
14	H	Cl		>120	>120	>120	>390
15	H	Cl		180	55	110	>390
16	H	Cl		>120	>120	ND	ND
17	H	Cl		>120	>120	ND	ND

[a] ND, Not done.

out by the 50% plaque reduction method as described previously (Suzutani et al., 1988; Kurihara et al., 1996). Briefly, confluent monolayers of MDCK cells grown in a 24-well microplate were infected with about 50 PFU of the A/PR/8/34 strain or the B/Gifu/2/73 strain per well. After 1.5 h of incubation at 37°C, the cell sheets were washed three times with MEM and overlaid with MEM containing 1 μg/ml trypsin (TPCK-treated, Type XIII; Sigma, St Louis, MO), a threefold

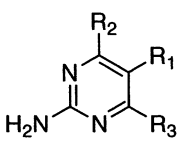
concentration of MEM-amino acids and vitamins, 1 mg/ml of glucose, 0.1 mg/ml of DEAE-dextran, 0.8% Agar Noble (Difco, Detroit, MI) and serially diluted test compound. The cells were incubated at 37°C for 2 days, fixed with formalin and stained with crystal violet after the removal of the overlay agar media by suction. The plaque counts were expressed as a percentage of the number obtained in control wells and were plotted to give dose-response lines, from which the effective concentration required to inhibit the virus plaque number by 50 or 90% (EC_{50} or EC_{90}, μM) was calculated.

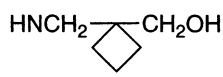
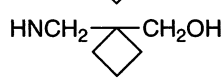
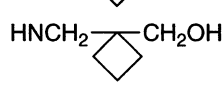
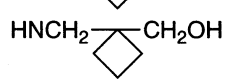
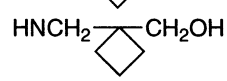
The antiviral activities of the test compounds against herpes viruses were also determined by the above method, except that HEL cell monolayers in 24-well microplates were used and the infected cells were overlaid with MEM containing 2% newborn calf serum, 0.5% methyl cellulose and serially diluted test compound. The incubation periods were 3 days for herpes simplex virus types 1 and 2, and 2 weeks for cytomegalovirus.

2.4. Assay of inhibitory effect of compounds on cell proliferation and cell viability

The assays of the inhibitory effects of the test compounds on cell proliferation and cell viability of MDCK cells were carried out in cells under different conditions: (1) cells not reaching confluency at day 2 after seeding of the cells in 24-well microplates at 2×10^4 cells/well (referred to as growing cells); (2) cells reaching complete confluency at day 2 after seeding of the cells at 4×10^5 cells/well (referred to as stationary cells). The former cell sheets were used for assaying the inhibitory effect of the compounds on the proliferation of growing cells, and the latter cell sheets were used for assaying the inhibitory effect of the compounds on the viability of stationary cells, i.e. cytotoxicity. At day 2 after seeding, the cells were

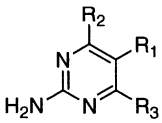
Table 3
Anti-influenza virus and anti-proliferative activity of pyrimidine derivatives

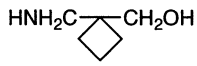
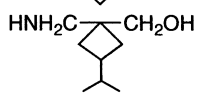
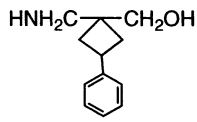
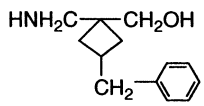
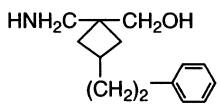
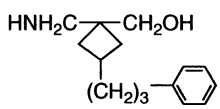
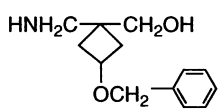
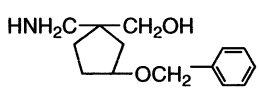
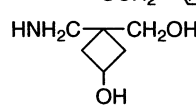
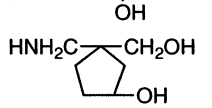
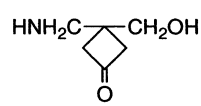
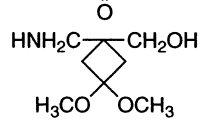


No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for growing MDCK cells
				Type A	Type B	
8	H	Cl		82–140	45–58	33–58
18	H	H		300	>480	ND[a]
19	H	OCH_3		46–120	50–55	42–100
20	H	CH_3		>140	>140	ND
21	H	OH		>130	>130	ND

[a] ND, Not done.

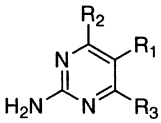
Table 4
Anti-influenza virus, anti-proliferative and cytotoxic activity of pyrimidine derivatives

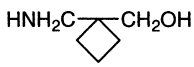
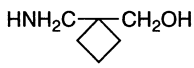
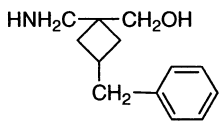
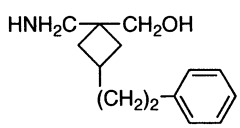
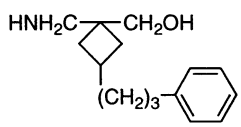
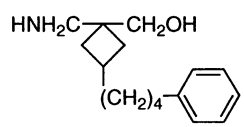
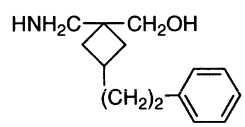
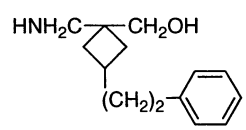
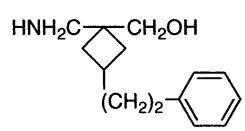
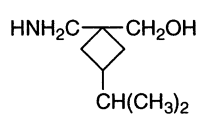


No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for MDCK cells	
				Type A	Type B	Growing	Stationary
8	H	Cl		82–140	45–58	33–58	>410
22	H	Cl		2.1–9.8	2.1–2.5	2.5–6.3	>105
23	H	Cl		3.5–6.0	2.8–3.8	5.0–8.8	>94
24	H	Cl		0.42–1.2	0.27–0.66	0.36–2.1	>90
25	H	Cl		0.29–1.2	0.35–0.63	0.81–0.92	>86
26	H	Cl		0.083–0.19	0.083–0.11	0.097–0.31	>83
27	H	Cl		13–20	7.2–16	1.7–4.3	>286
28	H	Cl		1.0–1.2	1.1–1.5	0.99–2.5	>83
29	H	Cl		>120	>120	>120	ND[a]
30	H	Cl		>110	>110	ND	ND
31	H	Cl		>120	>120	>390	ND
32	H	Cl		>99	>99	52	ND

[a] ND, Not done.

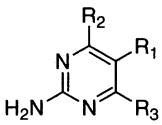
Table 5
Anti-influenza virus, anti-proliferative and cytotoxic activity of pyrimidine derivatives

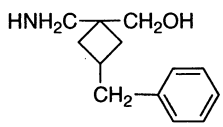
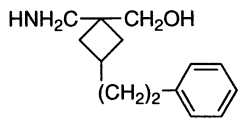
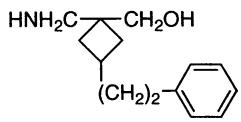
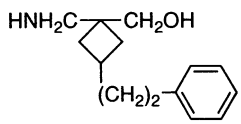
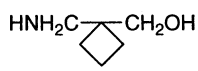
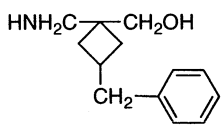
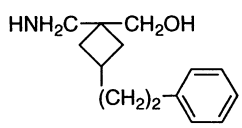
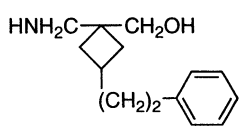
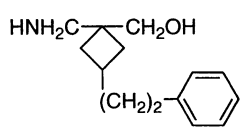


No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for MDCK cells	
				Type A	Type B	Growing	Stationary
8	H	Cl		82–140	45–58	33–58	>410
33	NH_2	Cl		35–43	9.7–47	18–20	>380
34	NH_2	Cl		0.12–0.17	0.17–0.23	0.46–0.92	>86
35	NH_2	Cl		0.055–0.19	0.14–0.25	0.22–0.33	>83
36	NH_2	Cl		0.016–0.027	0.016–0.027	0.024–0.053	>80
37	NH_2	Cl		0.10–0.13	0.026–0.051	0.077	>76
38	CH_2OH	Cl		0.53–0.80	0.16–0.80	0.13–0.27	ND[a]
39	CH=NOH	Cl		0.26–0.77	0.13–0.26	0.18–0.26	ND
40	CHO	Cl		6.7–8.0	7.5–9.6	9.6–12	ND
41	CHO	Cl		10–20	14–22	13–16	ND

[a] ND, Not done.

Table 6
Anti-influenza virus, anti-proliferative and cytotoxic activity of pyrimidine derivatives



No. of compound	R_1	R_2	R_3	EC_{50} (μM) for		IC_{50} (μM) for MDCK cells	
				Type A	Type B	Growing	Stationary
42	H	H		4.0–13	3.0–12	4.4–12	>100
43	H	H		4.5–16	9.0–14	11–18	>32
44	Br	H		0.026–0.20	0.13–0.26	0.46–0.77	>77
45	CH=NOH	H		0.39	0.25–0.51	1.1–2.8	>84
46	CH=NOH	H		22–56	14–32	40–72	ND[a]
47	NH_2	H		5.7–18	3.2–25	8.0–23	ND
48	NH_2	H		11–20	13–17	22–49	ND
49	CN	H		1.7–2.4	2.1–2.4	1.7–3.0	ND
50	CN	H		1.2–2.1	1.6–1.9	1.8–5.3	ND

[a] ND, Not done.

replenished with MEM-NCS10 containing an appropriate amount of the test compound. After incubation for an additional 2 days, the cells were dispersed by treatment with trypsin, and the viable cell numbers per well were counted by the trypan blue exclusion method. The concentration of compound that reduced the cell proliferation or cell viability by 50 or 90% (IC_{50} or IC_{90}, μM) was determined as described above.

3. Results

3.1. Structure–biological activity relationships

The EC_{50} or EC_{90} values for the influenza viruses, A/PR/8/34 (H1N1) strain and B/Gifu/2/73 strain, and the IC_{50} or IC_{90} values for the MDCK cells of the 2-amino-4-(ω-hydroxyalkylamino)pyrimidine derivatives are shown in Tables 1–7.

As shown in Table 1, the 2,4-diaminopyrimidines (compounds **1**, **2** and **3**) and the derivative in which aminopropanol was introduced into the 4-position of 2,4-diaminopyrimidine (compound **4**) had no anti-influenza virus activity. The compounds in which a dimethyl group and diethyl group were introduced into the β-position of the aminopropanol (compounds **5** and **6**, respectively) which are intermediate compounds for synthesizing compounds **7**–**11**, analogues of carbocyclic nucleosides, tended to have slightly increased anti-influenza virus activity. Earlier reports (Ichikawa et al., 1989; Nishiyama et al., 1989; Sakuma et al., 1991; Bisacchi et al., 1991) showed the antiviral activity of several carbocyclic compounds. The effects of substitutions at the β-position of aminoalkanol and the chain structure of the aminoalkanol of compound **4** on the biological activities were thus examined (Table 2). The derivatives in which cyclobutyl and cyclopentyl groups were introduced into the β-position of aminopropanol (compounds **8** and **9**, respectively) showed a potent antiviral activity against both influenza viruses A and B. These compounds also had an antiproliferative activity against growing MDCK cells to a degree similar to the antiviral activity, although they did not show cytotoxicity against stationary cells (Table 2). The cyclohexyl group-introduced derivative (compound **10**) showed a weak activity, whereas the cyclopropyl and oxetan group-introduced derivatives (compounds **7** and **11**, respectively) had no activity. The modifications of the chain structure of the aminoalkanol group of compound **8** (compounds **12**–**17**) showed decreased activity. The chlorine and methoxy group substitutions at the 6-position of the pyrimidine ring (compounds **8** and **19**, respectively) were required for antiviral activity (Table 3).

The effects of substitutions at the 3′-position of the β-cyclobutyl or β-cyclopentyl group on the antiviral and antiproliferative activities were then investigated in comparison with compound **8**. As shown in Table 4, the placement of an alkyl, aryl or araalkyl group at the 3′-position resulted in improved potency. In particular, the activity depended on the length of the alkyl group, in the order of the phenylpropyl (compound **26**), phenylethyl (compound **25**), phenylmethyl (compound **24**) and phenyl group (compound **23**). Again, these compounds showed an antiproliferative activity against the growing cells, but all these compounds had very low cytotoxicity for stationary cells. Although the introduction of a phenylmethyloxy group (compounds **27** and **28**) also produced antiviral activity, a complete loss of activity was observed with hydroxy, ketone, and dimethoxy substitutions at the 3′-position (compounds **29**–**32**, respectively). These results indicate that a bulky group introduced at the 3′-position of the β-cyclobutyl or β-cyclopentyl group was required for improved antiviral potency.

Experiments were then performed to determine the effect on antiviral activity of substituents at the 5-position of the pyrimidine ring. Table 5 shows that the introduction of an amino group (compounds **33**–**37**), hydroxymethyl group (compound **38**) or hydroxyiminomethyl group (compound **39**) at the 5-position increased the antiviral activity, and the compounds substituted with an amino group (compounds **34**–**37**) were found to be the most potent; e.g. compound **36** exhibited an average EC_{50} value of 0.01 μM for influenza viruses A and B. The compounds without substi-

Table 7
Anti-influenza virus, anti-proliferative and cytotoxic activity of selected pyrimidine derivatives in comparison with ribavirin

No. of compound	Average EC_{50} (μM) for		Average EC_{90} (μM) for		Average IC_{50} (μM) for MDCK cells		Average IC_{90} (μM) for growing MDCK cells	Antiviral index[a] for		Antiviral index[b] for	
	Type A	Type B	Type A	Type B	Growing	Stationary		Type A	Type B	Type A	Type B
24	0.81	0.47	0.93	0.75	1.23	>90	8.4	1.5	2.6	9.0	11.2
25	0.75	0.49	1.2	1.0	0.86	>86	8.9	1.1	1.8	7.4	8.9
26	0.14	0.11	0.20	0.17	0.19	>83	1.3	1.4	1.7	6.5	7.6
34	0.14	0.20	0.98	0.72	0.69	>86	3.2	4.9	3.5	3.3	4.4
35	0.11	0.19	0.28	0.55	0.28	>83	1.0	2.5	1.5	3.6	1.8
36	0.022	0.022	0.059	0.051	0.039	>80	0.20	1.8	1.8	3.4	3.9
37	0.12	0.039	0.33	0.18	0.077	>76	0.64	0.6	2.0	1.9	3.6
44	0.10	0.18	0.26	0.56	0.61	>77	8.4	6.1	3.4	32	15
45	0.39	0.37	1.8	0.70	2.0	>84	34	5.1	5.4	19	48
Ribavirin	6.1	3.3	13	7.0	8.2	>123	62	1.3	2.5	4.8	8.9

[a] Ratio of average IC_{50} for growing cells to average EC_{50} for virus.
[b] Ratio of average IC_{90} for growing cells to average EC_{90} for virus.

Table 8
Analytical data of novel compounds

No. of compound	Melting point (°C)	Recrystallization solvent	^{1}H-NMR (60 MHz)	Mass	
				EI (m/z)	CI (m/z)
4	160–163	EtOH	1.55–2.00(m, 2H), 3.40–3.71 (m, 4H), 6.33(s, 1H), 7.85(br, 3H), 9.31(br, 1H) $(CD_3)_2SO$	202	203
5	203–207	$CHCl_3$-MeOH	0.83(s, 6H), 3.08–3.15(m, 4H), 4.50(br, 1H), 5.79 (s, 1H), 6.13(s, 2H), 6.80–7.00(m, 1H) $(CD_3)_2SO$	230	231
6	185–187	$CHCl_3$	0.60–0.89(m, 6H), 1.05–1.28(m, 4H), 3.02–3.20(m, 4H), 4.60(t, 1H), 5.80(s, 1H), 6.25(s, 2H), 6.72–6.90 (m, 1H) $(CD_3)_2SO$	258	259
7	171–173	Acetone	0.42(s, 4H), 3.20–3.35(m, 4H), 4.52(t, 1H), 5.80(s, 1H), 5.97(s, 2H), 6.75–6.98(m, 1H) $(CD_3)_2SO$	228	229
8	192–194	Acetone	1.78(s, 6H), 3.32–3.42(m, 4H), 4.52–4.75(m, 1H), 5.80(s, 1H), 6.10(br, 2H), 6.77–6.95(m, 1H) $(CD_3)_2SO$	242	243
9	196–198	Acetone	1.38–1.70(m, 8H), 3.18–3.33(m, 4H), 4.72–4.95(m, 1H), 5.82(s, 1H), 6.07(s, 2H), 6.86–7.08(m, 1H) $(CD_3)_2SO$	256	257
10	221–222	Acetone	1.32(br, 10H), 3.12–3.24(m, 4H), 4.48–4.70(m, 1H), 5.84(s, 1H), 6.32(br, 2H), 6.85–7.10(m, 1H) $(CD_3)_2SO$	270	271
11	185–186	Acetone	3.55–3.64(m, 4H), 4.32(s, 4H), 4.70–4.90(m, 1H), 5.80(s, 2H), 6.18(br, 2H), 6.95–7.18(m, 1H) $(CD_3)_2SO$	244	245
12	233–235	MeOH	1.89–2.21(m, 6H), 3.65(d, 2H), 4.75–4.92(m, 1H), 5.70(s, 1H), 6.05(br, 2H), 7.00(br, 1H) $(CD_3)_2SO$	228	229
13	205–207	Acetone	1.55–2.12(m, 6H), 3.42(d, 2H), 5.20(s, 1H), 5.87 (s, 1H), 6.00(br, 2H), 6.70(br, 1H) $(CD_3)_2SO$	228	229
14	89–91	Et_2O	1.58–1.78(m, 8H), 3.00–3.40(m, 4H), 4.45(br, 1H), 5.74(s, 1H), 6.05(br, 2H), 6.68–6.88(m, 1H) $(CD_3)_2SO$	256	257
15	149–150	AcOEt	1.15–1.25(m, 3H), 1.68–1.88(m, 6H), 3.45(d, 2H), 3.60–3.95(m, 1H), 4.55(br, 1H), 5.50(s, 2H), 5.78 (s, 1H), 6.45–6.62(m, 1H) $CDCl_3+(CD_3)_2SO$	255	256
16	105–107	Et_2O	1.65–1.88(m, 8H), 3.38(d, 2H), 3.60–3.80(m, 2H), 5.23(br, 2H), 5.50–5.70(m, 1H), 5.75(s, 1H) $CDCl_3$	257	257
17	167–171	Acetone	1.76(s, 6H), 2.95(s, 3H), 3.22–3.50(m, 4H), 4.52–4.75 (m, 1H), 5.85(s, 1H), 6.32(s, 2H) $(CD_3)_2SO$	256	257
18	–		1.85(s, 6H), 3.40–3.78(m, 4H), 4.25(br, 1H), 6.33 (d, 1H), 7.58–7.70(m, 3H), 8.78–9.00(m, 1H) $(CD_3)_2SO$	208	209

Table 8 (Continued)

No. of compound	Melting point (°C)	Recrystallization solvent	^{1}H-NMR (60 MHz)	Mass	
				EI (m/z)	CI (m/z)
19	148–150	Acetone	1.75–1.78(m, 6H), 3.28–3.38(m, 4H), 3.73(s, 3H), 4.90(br, 1H), 5.13(s, 1H), 5.57(br, 2H), 6.28–6.48 (m, 1H) $(CD_3)_2SO$	238	239
20	176–177	Acetone	1.70(s, 6H), 1.95(s, 3H), 3.20–3.40(m, 4H), 4.70 (br, 1H), 5.59(s, 1H), 5.70(br, 2H), 6.42–6.68(m, 1H) $(CD_3)_2SO$	222	223
21	222–223	iso-PrOH	2.00(s, 6H), 3.38–3.60(m, 4H), 4.78(s, 1H), 4.80 (br, 1H), 6.40–6.60(m, 3H), 9.90(br, 1H) $(CD_3)_2SO$	224	225
22	183–185	Acetone	0.75(d, 6H), 1.35–1.90(m, 6H), 3.22–3.48(m, 4H), 4.60 (br, 1H), 5.80(d, 1H), 5.92(br, 2H), 6.78(br, 1H) $(CD_3)_2SO$	284	285
23	180–182	Acetone	1.75–2.40(m, 5H), 3.40–3.65(m, 4H), 4.25(br, 1H), 5.85(d, 1H), 6.12(br, 2H), 6.92–7.12(m, 1H), 7.28(s, 5H) $(CD_3)_2SO$	318	319
24	161–163	AcOEt	1.30–2.05(m, 5H), 2.67(br, 2H), 3.28–3.40(m, 4H), 4.65(br, 1H), 5.83(s, 1H), 6.00(br, 2H), 6.90(br, 1H), 7.20(s, 5H) $(CD_3)_2SO$	332[a]	333
25	167–169	Acetone	1.20–2.02(m, 7H), 2.50(t, 2H), 3.22–3.40(m, 4H), 4.70 (br, 1H), 5.50(s, 2H), 5.73(s, 1H), 6.38–6.68(m, 1H), 7.07(s, 5H) $CDCl_3+(CD_3)_2SO$	346[b]	347
26	154–156	Acetone	1.20–2.00(m, 9H), 2.45–2.70(m, 2H), 3.28–3.45(m, 4H), 4.15(br, 1H), 5.80(s, 1H), 6.17(br, 2H), 6.90(br, 1H), 7.20(s, 5H) $(CD_3)_2SO$	360[c]	361
27	137–138	Et_2O	1.55–2.28(m, 4H), 3.30–3.40(m, 4H), 3.85–4.20(m, 1H), 4.32(s, 2H), 4.70(br, 1H), 5.50(s, 2H), 5.78(s, 1H), 6.70(br, 1H), 7.27(s, 5H) $(CD_3)_2SO$	348	349
28	115–118	Et_2O	1.50–1.80(m, 6H), 3.25–3.48(m, 5H), 4.10(br, 1H), 4.47(s, 2H), 5.05(br, 2H), 5.40–5.60(m, 1H), 5.52(s, 1H), 7.32(s, 5H) $CDCl_3$	362	363
29	136–139		1.45–2.30(m, 4H), 3.30–3.40(m, 4H), 3.85–4.30(m, 1H), 4.60–4.88(m, 2H), 5.83(s, 1H), 6.10(s, 2H), 6.80–7.05 (m, 1H) $(CD_3)_2SO$	258	259
30	189–191	Acetone	1.45–1.70(m, 6H), 3.12–3.30(m, 4H), 4.20(br, 1H), 4.45–4.55 (m, 1H), 4.65–4.90(m, 1H), 5.78(s, 1H), 6.10(br, 2H), 6.98–7.18(m, 1H) $(CD_3)_2SO$	272	273
31	171–173	Acetone	2.80(s, 4H), 3.40–3.58(m, 4H), 4.85(t, 1H), 5.70(s, 1H), 6.23(s, 2H), 7.10(t, 1H) $CDCl_3+(CD_3)_2SO$	256	257
32	159–162	Acetone	1.85(s, 4H), 3.02(s, 6H), 3.22–3.35(m, 4H), 4.60(t, 1H), 5.70(s, 1H), 6.15(br, 2H), 6.80–7.00(t, 1H) $CDCl_3+(CD_3)_2SO$	302	303
33	226–229		1.80(s, 6H), 3.35–3.54(m, 4H), 3.70(br, 2H), 4.60(br, 1H), 5.50(br, 2H), 6.35–6.58(m, 1H) $(CD_3)_2SO$	257	258

Table 8 (Continued)

No. of compound	Melting point (°C)	Recrystallization solvent	^{1}H-NMR (60 MHz)	Mass	
				EI (m/z)	CI (m/z)
34	100–115		1.30–2.10(m, 5H), 2.62–2.80(m, 2H), 3.32–3.65(m, 4H), 4.50(s, 6H), 7.28(s, 5H) $CD_3OD + (CD_3)_2SO$	347[d]	348
35[e]	176–180		1.20–2.10(m, 7H), 2.42–2.68(m, 2H), 3.28–3.55(m, 4H), 4.31 (s, 6H), 7.18(s, 5H) $CD_3OD + (CD_3)_2SO$	361	362
36	–		1.10–2.14(m, 9H), 2.50–2.70(m, 2H), 3.50–3.82(m, 4H), 4.92(s, 6H), 7.25(s, 5H) CD_3OD	375[f]	376
37	125–127		1.21–1.30(m, 2H), 1.36–1.50(m, 4H), 1.59(q, 2H), 1.86–1.98 (m, 2H), 2.12–2.21 (m, 1H), 2.58(t, 2H), 3.35(s, 1H), 3.45(s, 2H), 3.49(s, 2H), 3.59(s, 1H), 7.09–7.15(m, 3H), 7.18–7.26(m, 2H) CD_3OD (300 MHz)	–[g]	–
38	167–170	Acetone	1.24–2.10(m, 7H), 2.38–2.62(m, 2H), 3.38–3.98(m, 4H), 4.48–4.92(m, 4H), 5.65(br, 2H), 6.52–6.72(m, 1H), 7.14(s, 5H) $CDCl_3 + (CD_3)_2SO$	376	377
39	154–156	Et_2O	1.22–2.00(m, 7H), 2.35–2.68(m, 2H), 3.22–3.62(m, 4H), 4.40(br, 1H), 5.68(br, 2H), 7.12(s, 5H), 8.40(s, 1H), 8.50–8.70(m, 1H), 10.45(s, 1H) $CDCl_3 + (CD_3)_2SO$	389	390
40	130–137	Et_2O	1.15–2.10(m, 7H), 2.42–2.65(m, 2H), 3.30–3.70(m, 4H), 4.00–4.25(m, 1H), 5.97(br, 2H), 7.18(s, 5H), 9.35–9.55 (m, 1H), 10.05(s, 1H) $CDCl_3$	374	375
41	156–158	Acetone	0.80(d, 6H), 1.30–1.94(m, 6H), 3.28–3.78(m, 4H), 4.42–4.68(m, 1H), 7.38(br, 2H), 9.18–9.48(m, 1H), 9.98(s, 1H) $(CD_3)_2SO$	312	313
42	Oil		1.38–2.05(m, 5H), 2.52–2.68(m, 2H), 3.32–3.45(m, 4H), 4.18(br, 1H), 6.28(d, 1H), 7.15(s, 5H), 7.50–7.70(m, 3H), 8.62–8.88(m, 1H) $(CD_3)_2SO$	298	299
43	–		1.32–2.05(m, 7H), 2.40–2.62(m, 2H), 3.40–3.62(m, 4H), 4.50(br, 1H), 6.30(d, 1H), 7.20(s, 5H), 7.50–7.70(m, 3H), 8.80(br, 1H) $(CD_3)_2SO$	312	313
44	156–160	Acetone	1.28–2.10(m, 7H), 2.40–2.55(m, 2H), 3.40–3.50(m, 4H), 4.85(br, 1H), 5.90(br, 2H), 6.45(t, 1H), 7.20(s, 5H) 7.78(s, 1H) $(CD_3)_2SO$	390[h]	391
45	170–176	Acetone	1.25–2.05(m, 7H), 2.35–2.60(m, 2H), 3.32–3.58(m, 4H), 4.60(br, 1H), 6.32(br, 2H), 7.20(s, 5H), 7.82(s, 1H) 8.00(s, 1H), 8.00–8.22(m, 1H) $(CD_3)_2SO$	355[i]	356
46	201–206	Acetone	1.80(s, 6H), 3.40–3.64(m, 4H), 4.15(br, 1H), 6.22(br, 2H), 7.76(s, 1H), 7.95(s, 1H), 8.00–8.15(m, 1H) $(CD_3)_2SO$	251	252
47	–		1.65–2.12(m, 5H), 2.50–2.82(m, 2H), 3.52–3.78(m, 4H), 4.78(s, 6H), 7.20(s, 5H), 7.30(s, 1H) $CD_3OD + (CD_3)_2SO$	313	314

Table 8 (Continued)

No. of compound	Melting point (°C)	Recrystallization solvent	^{1}H-NMR (60 MHz)	Mass	
				EI (m/z)	CI (m/z)
48	214–217	MeOH	1.30–2.12(m, 7H), 2.42–2.68(m, 2H), 3.50–3.78(m, 4H), 4.75(s, 6H), 7.17(s, 5H), 7.22(s, 1H) CD_3OD	327	328
49	184–189	MeOH	1.30–2.05(m, 7H), 2.40–2.68(m, 2H), 3.30–3.60(m, 4H) 4.75–4.95(m, 1H), 6.80(br, 2H), 7.18(s, 5H), 7.05–7.25 (m, 1H), 8.08(s, 1H)	337	338
50	156–159	MeOH	1.35–2.00(m, 7H), 2.12(s, 3H), 2.40–2.68(m, 3H) 3.45–3.55(m, 2H), 4.02–4.15(m, 2H), 5.50(br, 2H) 5.70–5.90(m, 1H), 7.12(s, 5H), 8.00(s, 1H)	379	380

[a] High-resolution mass spectrum (HRMS) calculated for $C_{17}H_{21}ClN_4O$ 332.1404, found 332.1401.
[b] HRMS calculated for $C_{19}H_{23}ClN_4O$ 346.1560, found 346.1562.
[c] HRMS calculated for $C_{19}H_{25}ClN_4O$ 360.1717, found 360.1714.
[d] HRMS calculated for $C_{17}H_{22}ClN_5O$ 347.1513, found 347.1510.
[e] Analysis calculated $C_{18}H_{24}ClN_5O$: C, 59.74; H, 6.69; N, 19.36; found: C, 59.93; H, 6.87; N, 19.37.
[f] HRMS calculated for $C_{19}H_{26}ClN_5O$ 375.1826, found 375.1823.
[g] HRMS calculated for $C_{20}H_{29}ClN_5O$ 390.2061, found 390.2068.
[h] HRMS calculated for $C_{18}H_{23}BrN_4O$ 390.1055, found 390.1053.
[i] HRMS calculated for $C_{19}H_{25}N_5O_2$ 355.2008, found 355.2011.

tution at the 5- and 6-position of the pyrimidine ring (compounds **42** and **43**) were significantly less potent than compounds **24** and **25**, which were substituted with chlorine at the 6-position of the pyrimidine ring (Tables 4 and 6). However, antiviral potency was restored by substitution with a halogen, hydroxyiminomethyl or cyano group at the 5-position (compounds **44**, **45**, **49** and **50**).

Table 7 shows the antiviral activities (EC_{50} and EC_{90}) and antiproliferative and cytotoxic activities (IC_{50} and IC_{90}) and the antiviral indices of selected compounds in comparison with the activities of ribavirin. It should be noted that 2-amino-4-(β-cyclobutyl-3′-phenylalkyl-γ-hydroxypropylamino)-5-amino-6-chloropyrimidines (compounds **34**–**37**) were found to have the most potent antiviral activity. The antiviral indices of these compounds were similar to that of ribavirin.

Compounds **8**, **9**, **11**, **15**, **19**, **21**, **22**, **24**, **25**, **27**–**31**, and **33**–**37** were selected from the viewpoint of structural similarity or dissimilarity and differences in anti-influenza virus activity, and were assessed for anti-herpesvirus activity in HEL cells. None of these compounds showed antiviral activity against herpes simplex virus type 1 (VR3 strain), type 2 (UW-268 strain) and cytomegalovirus (AD-169 strain) (data not shown).

4. Discussion

Amantadine and its analogue rimantadine have been available for many years for the prophylaxis and treatment of influenza virus A infection, but are not used for influenza virus B infection. The clinical use of these drugs has been limited by their side effects (Monto and Arden, 1992) and by the rapid emergence of resistant viral strains (Hayden et al., 1989). Ribavirin has also shown promising results in the treatment of influenza A and B by oral and inhalant administration (Van Voris and Newell, 1992). Several anti-influenza virus agents, which act through inhibition of viral neuraminidase (Von Itzstein et al., 1993; Thomas et al., 1994; Jedrzejas et al., 1995; Kim et al., 1997), cap-dependent endonuclease (Tomassini et al., 1994), *S*-adenosylhomocysteine hydrolase (Shigeta et al., 1992), fusion of the virus with an endosome/lysosome membrane (Nagai et al., 1995), or receptor binding of influenza virus (Tuzikov et al., 1997), have been reported, and, in particular, neuraminidase inhibitors have been further developed as anti-influenza drugs.

During our search for antivirally active guanosine analogues which could be expected to inhibit the PB1 subunit of influenza virus-specific RNA polymerase, we found that 2-amino-4-(ω-hydroxyalkylamino)pyrimidine had anti-influenza virus potency, although these compounds do not contain a guanine base. The 2-amino-4-(ω-hydroxyalkylamino)pyrimidine derivatives could be readily synthesized, and the nuclear magnetic resonance data for all these compounds, the high resolution mass spectrum data of the compounds selected in Table 7, or elemental analysis (compound **35**) gave satisfactory results (Table 8).

The derivatives in which a cyclobutyl group was introduced in the β-position of the aminoalkyl group, especially the cyclobutyl group substituted with a phenylalkyl group at the 3′-position (compounds **24**–**26** and **34**–**37**), showed an improved high anti-influenza virus activity depending on the number of carbon atoms per alkyl group. However, the activity was slightly decreased when the phenylalkyl at the 3′-position was a phenylbutyl group (compound **37**). The introduction of an amino, hydroxymethyl, or hydroxyiminomethyl group or bromine into the 5-position of the pyrimidine ring also increased the antiviral activity (Tables 5 and 6). The antiproliferative activity against the growing cells also increased in parallel with the increase of antiviral activity, although at present, the mechanisms of these antiviral and antiproliferative activities are unknown. All the compounds examined, however, had very low toxicity against stationary cells, suggesting that the anti-influenza virus activity of these compounds may not be due to non-specific cytotoxicity. In addition, the compounds showed no activity against herpes simplex virus types 1 and 2 and cytomegalovirus, again indicating that their anti-influenza virus activity must be specific.

In conclusion, compounds **34**–**37**, **44** and **45** have potent anti-influenza virus activity and very low toxicity against stationary cells. One may expect that these compounds would be efficacious

as an inhalation treatment for influenza virus infection. The efficacy of these compounds is now being assessed in an animal model, and further studies are expected to assess the mechanism of action and activity spectrum of these compounds against other strains of influenza viruses A and B and other RNA viruses.

Acknowledgements

We thank Dr Hiroshi Kida, Hokkaido University School of Veterinary Medicine, Sapporo, for kindly providing the B/Gifu/2/73 strain, and Tsuneo Nishi, ex-director of the Division of Drug Development, Nippon Shoji Co. Ltd., for encouraging us during this study. The excellent technical assistance of Chiaki Hatanaka is acknowledged.

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