8-Bromo-6-alkylamino-2-trifluoromethyl-9*H*-purines With *In Vitro* Activity Against Influenza A Virus

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Several derivatives of 8-bromo-6-dimethylamino-2-trifluoromethyl-9*H*-purine (**1**) were synthesized for structure-activity relationship studies of anti-influenza A virus activity. The 8-bromopurines were prepared by reaction of the anion of the 6-alkylamino-2-trifluoromethylpurines with *N*-bromosuccinimide. Several compounds had anti-influenza activity comparable to ribavirin, but no *in vivo* activity was observed.

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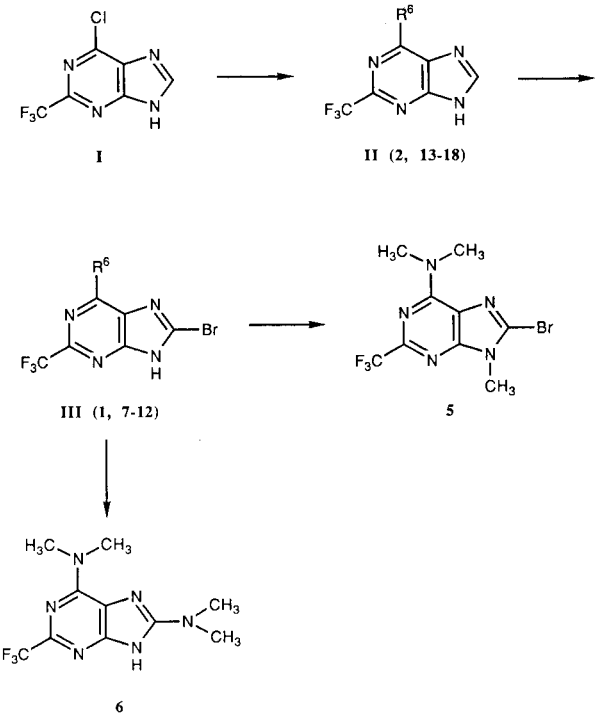
RNA viruses are the main causative factors of acute respiratory diseases, which are probably the most common cause of symptomatic human infections [1,2]. The influenza viruses belong to the family *Orthomyxoviridae*, which is one of five taxonomically distinct families of RNA viruses that are causative agents for human respiratory disease [3]. Of the three types of influenza virus, types A and B have been associated with significant increases in mortality during epidemics. Immunization against influenza has been recommended for high-risk groups, and antiviral chemotherapy is available for the treatment and prophylaxis of all influenza A infections [2]. Whereas amantadine and rimantadine are only useful for treatment of influenza A infections, ribavirin has reportedly been effective against both influenza A and B when administered to patients by inhalation through a face mask [4,5]. Thus, there is room for improvement of the physican's armamentarium of drugs for treatment of influenza virus infections [2].

As part of an ongoing program directed to the discovery and development of antiviral agents [6-12], we tested a variety of compounds for *in vitro* activity against influenza virus. The tri-substituted purine **1** was discovered to have *in vitro* activity against influenza A virus, which was comparable to ribavirin but approximately 9-fold weaker than amantadine. A variety of analogues were synthesized to study the structure-activity relationship (SAR) of **1** and its anti-influenza A activity; the results are reported herein.

Chemistry.

The intermediate 6-alkylamino-2-trifluoromethylpurines **II** were prepared by amination of **I** [12] with the appropriate amine (Scheme 1). Initial attempts to brominate **2** met with little success. For example, bromination of **2** with excess bromine in glacial acetic acid at steam bath temperature gave a three-component mixture. Use of a large excess of bromine with sodium acetate-buffered tetrahydrofuran-acetic acid also gave a mixture. However, reaction of the anion of **2** [12] with *N*-bromosuccinimide in hot dimethylformamide provided the 8-bromopurine **1** in 59% yield. This method was also applied to the 2-trifluoromethylpurines **13-18** to provide the 8-bromopurines in unoptimized yields that varied from 20 to 60% (Table I).

Scheme 1

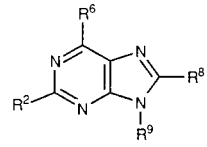


Compound **1** was alkylated with methyl iodide in the presence of potassium carbonate to give **5** in 25% yield. That compound **5** was the 9-isomer was evident from the similarity of its uv spectrum with the 9-benzyl analogue [13]. The 8-bromo group of **1** was readily displaced with dimethyl amine to give **6**.

Biological Results and Discussion.

The compounds listed in Table II were tested initially in

Table I
Physical Properties of Purines



Compound No.	R^2	R^6	R^8	R^9	Method [a]	Yield, %	MP, °C	Molecular Formula	Analyses % Calcd. (Found) C	H	N
1	CF_3	$N(CH_3)_2$	Br	H	A	59 [b]	228-230	$C_8H_7BrF_3N_5$	30.99	2.28	22.59
									(30.93)	(2.32)	(22.49)
2	CF_3	$N(CH_3)_2$	H	H	B [a]						
3	H	$N(CH_3)_2$	Br	H	A [c]	33 [d]	245-247 [e]	$C_7H_8BrN_5$	34.73	3.33	28.93
									(34.78)	(3.33)	(28.89)
4	Cl	$N(CH_3)_2$	Br	H	A [c]	61 [f]	283-286 dec	$C_7H_7BrClN_5$	30.41	2.55	25.33
									(30.53)	(2.56)	(25.28)
5	CF_3	$N(CH_3)_2$	Br	CH_3	C	25 [b]	151-152	$C_9H_9BrF_3N_5$	33.35	2.80	21.61
									(33.51)	(2.87)	(21.69)
6	CF_3	$N(CH_3)_2$	$N(CH_3)_2$	H	B [g]	44 [b]	203-204	$C_{10}H_{13}F_3N_6$	43.80	4.78	30.64
									(43.83)	(4.82)	(30.59)
7	CF_3	$NHCH_3$	Br	H	A [h]	20 [b]	249-250.5	$C_7H_5BrF_3N_5$	28.40	1.70	23.66
									(28.46)	(1.71)	(23.65)
8	CF_3	$N(CH_3)CH_2CH_3$	Br	H	A [i]	35 [b]	170-172	$C_9H_9BrF_3N_5$	33.35	2.80	21.61
									(33.29)	(2.83)	(21.60)
9	CF_3	$N(CH_3)CH_2CH_2CH_3$	Br	H	A [i]	41 [j]	155-157	$C_{10}H_{11}BrF_3N_5$	35.52	3.28	20.71
									(35.48)	(3.30)	(20.69)
10	CF_3	$N(CH_3)$-*c*-C_3H_5	Br	H	A [i]	58 [b]	194-196	$C_{10}H_9BrF_3N_5$	35.73	2.70	20.84
									(35.61)	(2.74)	(20.77)
11	CF_3	$N(CH_3)CH_2C_6H_5$	Br	H	A [k]	43 [b]	215-217	$C_{14}H_{11}BrF_3N_5$	43.54	2.87	18.14
									(43.48)	(2.93)	(18.12)
12	CF_3	$N(CH_2CH_3)_2$	Br	H	A	60 [j]	178-181	$C_{10}H_{11}BrF_3N_5$	35.52	3.28	20.71
									(35.65)	(3.32)	(20.70)
13	CF_3	$NHCH_3$	H	H	B [l]	63	350-352 [m]	$C_7H_6F_3N_5$	38.71	2.78	32.25
									(38.87)	(2.82)	(32.21)
14	CF_3	$N(CH_3)CH_2CH_3$	H	H	B [n]	80	244-246 [o]	$C_9H_{10}F_3N_5$	44.08	4.11	28.56
									(44.15)	(4.11)	(28.54)
15	CF_3	$N(CH_3)CH_2CH_2CH_3$	H	H	B [p]	76	205-208 [q]	$C_{10}H_{12}F_3N_5$	46.33	4.67	27.02
									(46.34)	(4.71)	(26.99)
16	CF_3	$N(CH_3)$-*c*-C_3H_5	H	H	B [r]	66	245-247 [o]	$C_{10}H_{10}F_3N_5$	46.70	3.92	27.23
									(46.70)	(3.96)	(27.22)
17	CF_3	$N(CH_3)CH_2C_6H_5$	H	H	B [s]	72 [q]	224-227	$C_{14}H_{12}F_3N_5$	54.72	3.94	22.79
									(54.78)	(3.98)	(22.77)
18	CF_3	$N(CH_2CH_3)_2$	H	H	B [t]	39 [o]	180-182	$C_{10}H_{12}F_3N_5$	46.33	4.67	27.02
									(46.28)	(4.68)	(26.97)

[a] Method B: see preparation of 2 in reference [12]. [b] Recrystallized from hexane-ethyl acetate. [c] The reaction was heated to 100°; the extraction was done with ethyl acetate. [d] Recrystallized from methanol-water . [e] MP 227-228°C reported for this compound by L. M. Roitshtein, Kh. L. Muravich-Aleksandr, and A. V. El'tsov, *Zh. Obshch. Khim.*, **39**, 2125 (1969) by a different method. [f] Recrystallized from ethyl acetate. [g] The reaction was done in a stainless steel, glass-lined reaction vessel at 105° for 18 hours using 2.2 *M* dimethylamine in ethanol. [h] The reaction was done at ambient temperature for 3 hours. [i] The reaction was heated to 100°. [j] Recrystallized from hexane. [k] The reaction was heated to 110° for 1.25 hours. [l] The reaction was heated 0.5 hours on a steam bath with 40% aqueous methylamine in ethanol. [m] Recrystallized from ethyl acetate-ethanol. [n] The reaction was performed with 10 parts of *N*-methylethylamine in ethanol at ambient temperature for 42 hours. [o] Recrystallized from ethanol-water. [p] The reaction was performed with 10 parts of *N*-methylpropylamine in ethanol at ambient temperature for 18 hours. [q] Recrystallized from ethyl acetate-hexane. [r] The reaction was performed with 2 parts of *N*-methylcyclopropylamine and 1.5 parts of triethylamine in ethanol at 60° for 18 hours. [s] The reaction was performed with 10 parts of *N*-methylbenzylamine in ethanol at ambient temperature for 18 hours. [t] The reaction was performed with 10 parts of diethylamine in ethanol at 50° for 18 hours.

a plaque inhibition assay against influenza A/Sweden/3/50 (HINI) using Madin-Darby canine kidney (MDCK) cells [14]. Activity results were recorded as inactive (−), slightly active (±) or active (+) at 50 μg per disc. For three compounds the 50% inhibitory concentration (IC_{50}) was measured with the plaque reduction assay [15].

Table II

Activity of Purines Against Influenza A/Sweden/3/50 [HINI]

Compound Number	Plaque [a,b] Inhibition	IC_{50}, μ*M*
1	+	15
2	-	
3	-	
4	- (S)	
5	-	
6	-	
7	-	
8	± (S)	
9	± (T)	
10	± (S)	25 [c]
11	± (T)	
12	± (S)	12.5 [d]
amantidine	+	1.6
ribavirin	+	30

[a] For methodolgy see references 14-17; + = active at 50 μg per disc, ± = slight activity, - = inactive. [b] The *in vitro* toxicity was assessed by observing the cells in the plaque inhibition assay; T = toxic, S = slight toxicity at 50 μg per disc or at concentration footnoted. [c] Slight toxicity at 50 μ*M*. [d] Slight toxicity at 25 μ*M*.

The trisubstituted 2-trifluoromethylpurine **1** was active against influenza A virus with an IC_{50} of 15 μ*M* under conditions where ribavirin and amantadine had IC_{50}'s of 30 and 1.6 μ*M*, respectively. Removal of the 8-bromo or 2-trifluoromethyl substituents to give **2** and **3** resulted in loss of activity. The 2-chloro analogue **4** was also inactive in the plaque inhibition test. Substitution at the 9-position with methyl (see **5**) or at the 8-position with dimethylamino (see **6**) led to analogues inactive at 50 μg per disc.

Although anti-influenza A activity was incompatible with substituent variation on **1** at the 2-, 8- or 9-positions, some substituent variation at the 6-position led to activity. The monomethylamino analogue **7** was not active, but the disubstituted amino derivatives **8-12** had slight activity in the plaque inhibition assay. The activities of **10** and **12** were quantitated in the plaque reduction assay and had IC_{50}'s of 25 and 12.5 μ*M*, respectively.

Compound **1** was tested for *in vivo* anti-influenza activity in mice using influenza A/Sweden/3/50 [HINI], which had been adapted to mice by serial passage in the mouse lung [16]. Groups of five mice were infected intranasally with virus, and the growth of virus in the lung was determined on 10% lung suspensions by plaque titrations in MDCK cells [17]. No significant activity was observed when 100 mg/kg of **1** was administered s.c. twice a day for two days. In this test system 100 mg/kg amantadine gave a 100-fold decrease in virus titer.

A method for preparation of 8-bromo derivatives of 6-alkylamino-2-trifluoromethylpurines was developed. Although several 8-bromo-6-alkylamino-2-trifluoromethyl-9*H*-purines had *in vitro* anti-influenza A activity comparable to ribavirin, no significant *in vivo* activity was observed for **1** in a mouse model at 100 mg/kg.

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and are uncorrected. The nmr spectra were recorded on a Varian XL-100-15-FT or a Varian FT-80A spectrometer using tetramethylsilane as an internal standard. The uv absorption spectra were measured on a Cary 118 UV-vis spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on thin-layered chromatography (tlc). The tlc's were developed on Whatman 200 μ MK6F plates of silica gel with fluorescent indicator. Preparative flash chromatography was performed on Silica Gel 60 (40-63 μm, E. Merck No. 9385). Elemental analyses were performed by Atlantic Microlab, Inc.

Method A. 8-Bromo-6-dimethylamino-2-trifluoromethyl-9*H*-purine (**1**).

Dimethylformamide (5 ml) was added to sodium hydride (60.2% in mineral oil) (0.103 g, 2.59 mmoles), which had been washed with pentane (2 x 5 ml). 6-Dimethylamino-2-trifluoromethyl-9*H*-purine (**2**) [12] (0.500 g, 2.16 mmoles) was added, and the mixture was stirred for 0.5 hours. *N*-Bromosuccinimide (0.461 g, 2.59 mmoles) was added to the mixture, and the reaction was heated to 120° for 0.5 hours. The reaction mixture was poured into an ice-water slurry (60 ml) and acidified to *p*H 4 with glacial acetic acid. The solids were collected by filtration. The filtrate was extracted with ether (2 x 50 ml), and the extract was combined with a solution of the solids in ether (50 ml). The combined solution was washed with water (2 x 75 ml), brine (50 ml), dried (sodium sulfate), and spin evaporated *in vacuo*. The solid residue was co-evaporated with dichloromethane (50 ml) to give 0.667 g (100%) of a solid residue, which was dissolved in 50 ml of ethyl acetate-hexane (1:2). This solution was applied to a column of Silica Gel 60, which was wetted with ethyl acetate-hexane (1:2). The column was eluted with ethyl acetate-hexane (1:2), and fractions containing the major component were combined and spin evaporated *in vacuo*. Recrystallization from hexane-ethyl acetate gave 0.392 g (59%) of **1**, mp 228-230°; tlc, ethyl acetate-cyclohexane (1:2), one spot with R_f = 0.52; uv (0.1 *N* hydrochloric acid + 10% ethanol): λ max 277.5 nm; (*p*H 7.0 buffer + 10% ethanol): λ max 284.5 nm; (0.1 *N* sodium hydroxide + 10% ethanol): λ max 285.5 nm; ^{1}H nmr (DMSO-d_6): δ 3.44 (br s, 6 H, $N(CH_3)_2$); ms: m/e 309, 311 (M^+), 294, 296 ($M-CH_3$)$^+$, 280, 282 ($M-NCH_3$)$^+$, 230 ($M-Br$)$^+$, 69 (CF_3)$^+$.

Anal. Calcd. for $C_8H_7BrF_3N_5$: C, 30.99; H, 2.28; N, 22.59. Found: C, 30.93; H, 2.32; N, 22.49.

Method C. 8-Bromo-6-dimethylamino-9-methyl-2-trifluoromethyl-9*H*-purine (**5**).

Methyl iodide (0.46 g, 3.2 mmoles) was added to a stirred mixture of **1** (0.310 g, 1.00 mmole), anhydrous potassium carbonate (0.200 g, 1.45 mmoles), and dimethyl sulfoxide (5 ml). The reaction mixture was heated at 70° for 1.5 hours and then poured over ice-water (100 ml). The mixture was extracted with dichloromethane (3 x 30 ml). The extracts were washed with water (5 x 20 ml) and spin evaporated *in vacuo*. The residue was dissolved in ethyl acetate, added to Silica Gel 60, and spin evaporated *in vacuo*. The residual solids were introduced onto a column of Silica Gel 60 wetted with ethyl acetate-cyclohexane (1:2). The column was eluted with the same solvent, and fractions containing the major component were combined and spin evaporated *in vacuo*. Recrystallization from cyclohexane-ethyl acetate gave 0.080 g (25%) of **5**, mp 151-152°; uv (0.1 *N* hydrochloric acid + 10% ethanol): λ max 279 nm; (0.1 *N* sodium hydroxide + 10% ethanol): λ max 278.5 nm; ^{1}H nmr (DMSO-d_6): δ 3.72 (s, 3 H, CH_3), 3.44 (br s, 6 H, $N(CH_3)_2$).

Anal. Calcd. for $C_9H_9BrF_3N_5$: C, 33.35; H, 2.80; N, 21.61. Found: C, 33.51; H, 2.87; N, 21.69.

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