

James L. Kelley* and James A. Linn

Division of Organic Chemistry, Burroughs Wellcome Co.,
Research Triangle Park, NC 27709

Margaret Tisdale

Wellcome Research Laboratories, Langley Court,
Beckenham, Kent BR3 3BS U. K.

Received January 26, 1990

Several derivatives of 8-bromo-6-dimethylamino-2-trifluoromethyl-9H-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.

J. Heterocyclic Chem., **27**, 1505 (1990).

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 physician's armamentarium of drugs for treatment of influenza virus infections [2].

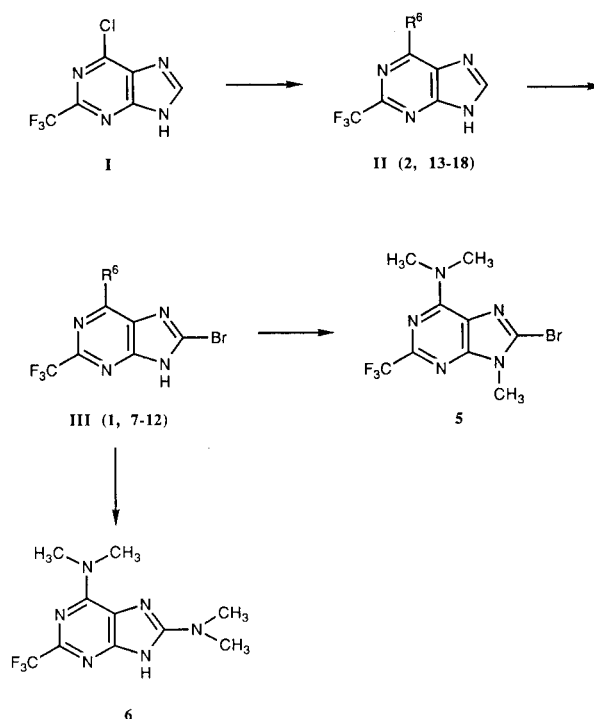
As part of an ongoing program directed to the discovery and development of antiviral agents [6-12], we tested a variety of compounds for *in vitro* activity against influenza virus. The tri-substituted purine **1** was discovered to have *in vitro* activity against influenza A virus, which was comparable 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 di-

methylformamide 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

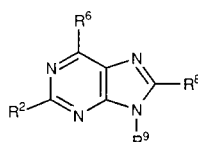


Compound **1** was alkylated with methyl iodide in the presence of potassium carbonate to give **5** in 25% yield. That compound **5** was the 9-isomer was evident from the similarity of its uv spectrum with the 9-benzyl analogue [13]. The 8-bromo group of **1** was readily displaced with dimethyl amine 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 ₈ H ₇ BrF ₃ N ₅	30.99 (30.93)	2.28 (2.32)	22.59 (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 ₇ H ₇ BrN ₅	34.73 (34.78)	3.33 (3.33)	28.93 (28.89)
4	Cl	N(CH ₃) ₂	Br	H	A [c]	61 [f]	283-286 dec	C ₇ H ₇ BrClN ₅	30.41 (30.53)	2.55 (2.56)	25.33 (25.28)
5	CF ₃	N(CH ₃) ₂	Br	CH ₃	C	25 [b]	151-152	C ₉ H ₉ BrF ₃ N ₅	33.35 (33.51)	2.80 (2.87)	21.61 (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 ₃ N ₆	43.80 (43.83)	4.78 (4.82)	30.64 (30.59)
7	CF ₃	NHCH ₃	Br	H	A [h]	20 [b]	249-250.5	C ₇ H ₅ BrF ₃ N ₅	28.40 (28.46)	1.70 (1.71)	23.66 (23.65)
8	CF ₃	N(CH ₃)CH ₂ CH ₃	Br	H	A [i]	35 [b]	170-172	C ₉ H ₉ BrF ₃ N ₅	33.35 (33.29)	2.80 (2.83)	21.61 (21.60)
9	CF ₃	N(CH ₃)CH ₂ CH ₂ CH ₃	Br	H	A [i]	41 [j]	155-157	C ₁₀ H ₁₁ BrF ₃ N ₅	35.52 (35.48)	3.28 (3.30)	20.71 (20.69)
10	CF ₃	N(CH ₃)- <i>c</i> -C ₃ H ₅	Br	H	A [i]	58 [b]	194-196	C ₁₀ H ₉ BrF ₃ N ₅	35.73 (35.61)	2.70 (2.74)	20.84 (20.77)
11	CF ₃	N(CH ₃)CH ₂ C ₆ H ₅	Br	H	A [k]	43 [b]	215-217	C ₁₄ H ₁₁ BrF ₃ N ₅	43.54 (43.48)	2.87 (2.93)	18.14 (18.12)
12	CF ₃	N(CH ₂ CH ₃) ₂	Br	H	A	60 [j]	178-181	C ₁₀ H ₁₁ BrF ₃ N ₅	35.52 (35.65)	3.28 (3.32)	20.71 (20.70)
13	CF ₃	NHCH ₃	H	H	B [l]	63	350-352 [m]	C ₇ H ₆ F ₃ N ₅	38.71 (38.87)	2.78 (2.82)	32.25 (32.21)
14	CF ₃	N(CH ₃)CH ₂ CH ₃	H	H	B [n]	80	244-246 [o]	C ₉ H ₁₀ F ₃ N ₅	44.08 (44.15)	4.11 (4.11)	28.56 (28.54)
15	CF ₃	N(CH ₃)CH ₂ CH ₂ CH ₃	H	H	B [p]	76	205-208 [q]	C ₁₀ H ₁₂ F ₃ N ₅	46.33 (46.34)	4.67 (4.71)	27.02 (26.99)
16	CF ₃	N(CH ₃)- <i>c</i> -C ₃ H ₅	H	H	B [r]	66	245-247 [o]	C ₁₀ H ₁₀ F ₃ N ₅	46.70 (46.70)	3.92 (3.96)	27.23 (27.22)
17	CF ₃	N(CH ₃)CH ₂ C ₆ H ₅	H	H	B [s]	72 [q]	224-227	C ₁₄ H ₁₂ F ₃ N ₅	54.72 (54.78)	3.94 (3.98)	22.79 (22.77)
18	CF ₃	N(CH ₂ CH ₃) ₂	H	H	B [t]	39 [o]	180-182	C ₁₀ H ₁₂ F ₃ N ₅	46.33 (46.28)	4.67 (4.68)	27.02 (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 (H1N1) using Madin-Darby canine kidney (MDCK) cells [14]. Activity results were recorded as inactive (-), slightly active (\pm) or active (+) at 50 μ g 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 [H1N1]

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

[a] For methodology see references 14-17; + = active at 50 μ g per disc, \pm = 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 μ g 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 amantidine 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 μ g 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 [H1N1], 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 amantidine 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-9H-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 μ m, E. Merck No. 9385); Elemental analyses were performed by Atlantic Microlab, Inc.

Method A. 8-Bromo-6-dimethylamino-2-trifluoromethyl-9H-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-9H-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 pH 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; (pH 7.0 buffer + 10% ethanol): λ max 284.5 nm; (0.1 *N* sodium hydroxide + 10% ethanol): λ max 285.5 nm; ¹H nmr (DMSO-*d*₆): δ 3.44 (br s, 6 H, N(CH₃)₂); ms: m/e 309, 311 (M⁺), 294, 296 (M-CH₃)⁺, 280, 282 (M-NCH₃)⁺, 230 (M-Br)⁺, 69 (CF₃)⁺.

Anal. Calcd. for C₈H₈BrF₃N₂: 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-9H-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; ¹H nmr (DMSO-d₆): δ 3.72 (s, 3 H, CH₃), 3.44 (br s, 6 H, N(CH₃)₂).

Anal. Calcd. for C₉H₉BrF₃N₃: C, 33.35; H, 2.80; N, 21.61. Found: C, 33.51; H, 2.87; N, 21.69.

Acknowledgement.

We thank Dr. B. S. Hurlbert and his staff for some of the NMR spectra. We acknowledge the assistance of Ms. T. Cozart, S. Paris, J. Appleton and D. Tabon in preparation of the manuscript and thank Mr. A. Jones for proofreading the manuscript.

REFERENCES AND NOTES

- [1] L. J. Anderson, P. A. Patriarca, J. C. Hierholzer and G. R. Noble, *Med. Clin. North Am.*, **67**, 1009 (1983).
- [2] J. L. Kelley, *Annu. Rep. Med. Chem.*, **19**, 117 (1984).
- [3] E. H. Lennette, *Bull. W.H.O.*, **59**, 305 (1981).
- [4] V. Knight, H. W. McClung, S. Z. Wilson, B. K. Waters, J. M. Quarles, R. W. Cameron, S. E. Greggs, J. M. Zerwas and R. B. Couch, *Lancet*, **2**, 945 (1981).
- [5] H. W. McClung, V. Knight, B. E. Gilbert, S. Z. Wilson, J. M. Quarles and G. W. Divine, *J. Am. Med. Assoc.*, **249**, 2671 (1983).
- [6] G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, *Proc. Natl. Acad. Sci. U. S. A.*, **74**, 5716, (1977).
- [7] H. J. Schaeffer, Nucleosides, Nucleotides and Their Biological Application, J. L. Rideout, D. W. Henry and L. M. Beacham III, eds, Academic Press, New York, 1983, p 1.
- [8] D. J. Bauer, J. W. T. Selway, J. F. Batchelor, M. Tisdale, I. C. Caldwell and D. A. B. Young, *Nature*, **292**, 369 (1981).
- [9] H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. Nusinoff-Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry and S. Broder, *Proc. Natl. Acad. Sci. U. S. A.*, **82**, 7096 (1985).
- [10] J. L. Kelley, J. A. Linn, M. P. Krochmal and J. W. T. Selway, *J. Med. Chem.*, **31**, 2001 (1988).
- [11] J. L. Kelley, J. A. Linn and J. W. T. Selway, *J. Med. Chem.*, **32**, 218 (1989).
- [12] J. L. Kelley, J. A. Linn and J. W. T. Selway, *J. Med. Chem.*, **32**, 1757 (1989).
- [13] J. L. Kelley, J. A. Linn and J. W. T. Selway, in preparation.
- [14] J. S. Oxford, K. A. Callow, T. Corcoran and A. S. Beare, *Arch. Virol.*, **74**, 227 (1982).
- [15] J. L. Kelley, C. A. Miller, J. W. T. Selway and H. J. Schaeffer, *Eur. J. Med. Chem.*, **23**, 319 (1988).
- [16] M. Tisdale and D. J. Bauer, *J. Antimicrobial Chemother.*, **1** (Suppl), 55 (1975).
- [17] M. Tisdale and D. J. Bauer, *Ann. N. Y. Acad. Sci.*, **284**, 254 (1977).