Inhibition of HSV-1 Replication. The effect of drugs on virus replication was measured as described previously[25] 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 earlier[26] 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 ^{3}H-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 [^{3}H]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 [^{3}H]thymidine, incorporation of ^{3}H-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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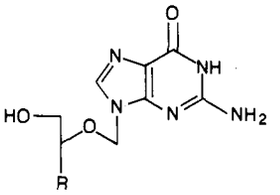
Synthesis and Antiviral Activity of Novel N-Substituted Derivatives of Acyclovir†

Jerzy Boryski,‡ Bozenna Golankiewicz,*,‡ and Erik De Clercq*,§

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium. *Received November 5, 1987*

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 properties[15] (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 = CH_2OH

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.

‡ Polish Academy of Sciences.
§ Rega Institute for Medical Research.

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Table I. 90-MHz ^1H NMR Spectral Data in DMSO-d_6[a]

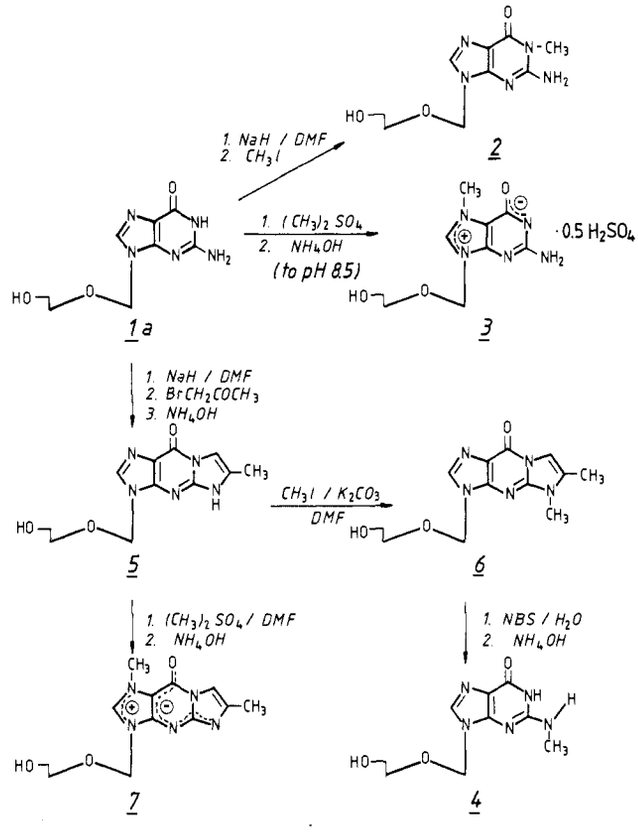
compd	chemical shifts of the protons in ppm (δ) from TMS							
	N-1-H	N-2-H	8-H	NCH_2O	OH	CH_2CH_2	isopropeno	NCH_3
1a (ACV)	10.65 (brs, 2)	6.51 (brs, 2)	7.81 (s, 1)	5.35 (s, 2)	4.67 (brt, 1)	3.49, 3.46 (2 s, 4)		
2		7.06 (brs, 2)	7.82 (s, 1)	5.35 (s, 2)	4.65 (brm, 1)	3.48, 3.45 (2 s, 4)		3.32 (s, 3)
3		6.72 (brs, 2)	9.17 (s, 1)	5.50 (s, 2)	n	3.54, 3.47 (2 s, 4)		4.02 (s, 3)
4	10.60 (brs, 1)	6.37 (q, 1)	7.82 (s, 1)	5.39 (s, 2)	4.68 (brt, 1)	3.51 (br, 4)		2.82 (d, 3)
5		12.42 (brs, 1)	8.03 (s, 1)	5.49 (s, 2)	4.69 (br, 1)	3.50 (br, 4)	7.36 (d, 1), 2.27 (d, 3)	
6			8.05 (s, 1)	5.52 (s, 2)	4.67 (brt, 1)	3.55 (m, 4)	7.46 (d, 1), 2.32 (d, 3)	3.59 (s, 3)
7			9.31 (s, 1)	5.62 (s, 2)	n	3.58 (m, 4)	7.29 (d, 1), 2.22 (d, 3)	4.12 (s, 3)

[a] Signals are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; or n, if no firm assignment could be made. Following the observed multiplicities are the numbers of protons as estimated by integration.

(dThd) kinase.[19] The resulting acyclovir monophosphate is further phosphorylated to the triphosphate form by cellular kinases.[20,21] Acyclovir triphosphate interferes with viral DNA synthesis through both a direct inhibitory effect on the viral DNA polymerase and chain-terminating effect (following incorporation at the 3′-end[22-24]). The virus-encoded deoxythymidine kinase has apparently less stringent requirements than the normal mammalian cell enzymes, but while the viral enzyme readily recognizes several acyclic analogues of guanosine as substrate, it does not tolerate much variations in the guanine moiety.[25]

The antiviral properties of a series of acyclovir derivatives with modifications in the heterocyclic base have recently been the subject of a systematic investigation.[26]

Scheme I



These modifications included monocyclic, bicyclic, and tricyclic congeners. None of these compounds exhibited a significant antiherpetic activity.[26]

In delineating the structural features of the base moiety of acyclovir that are crucial for its antiviral activity, it seemed of interest to assess the importance of the nitrogen centers. In this perspective, several new acyclovir derivatives (**2–7**) were prepared in which one or more nitrogen centers were blocked by methylation or by incorporation into an additional ring.

Chemistry

The N-substituted acyclovir derivatives were prepared as outlined in Scheme I. Monomethylation of acyclovir **1a** at positions 1, 7, and N-2 gave compounds **2–4**, respectively. Simultaneous blocking of the positions 1 and N-2, by linking them together via an isopropeno group, provided the tricyclic derivative **5**.[27] The latter was further methylated to give tri-N-substituted compounds **6** and **7**.

The procedures used for the preparation of the acyclovir derivatives mostly followed that previously described for

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(27) Systematic name: 5,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-methyl-9-oxo-5*H*-imidazo[1,2-*a*]purine.

Table II. Ultraviolet Spectral and Thin-Layer Chromatography Data

compd	H_2O λ_{max}, nm (ϵ)[b]	R_f values × 100 in system[a] A	B	C
1a (ACV)	253 (14500), 269 (sh, 11700)	65	46	09
2	255 (12500), 270 (sh, 11800)	69	43	25
3	255 (12500), 281 (sh, 8500)		23	01
4	253 (13400), 279 (sh, 8400)	65	50	35
5	231 (27300), 285 (10000)	72	49	45
6	233 (27400), 288 (12100)	71	43	60
7	230 (25300), 276 (8300), 299 (6700)		30	03

[a] See the Experimental Section. [b] Shoulder, sh.

guanosine.[28-30] Methylation of **1a** at position 1 to give **2** in 56% yield was achieved when an anion of **1a** was generated with sodium hydride and reacted with methyl iodide in dimethylformamide. The use of potassium carbonate and dimethyl sulfoxide, as described in ref 31, resulted in a product that proved difficult to purify.

In the absence of a base, acyclovir in dimethylformamide reacted with dimethyl sulfate at position 7. Careful adjustment of the reaction mixture to pH 8.5 resulted in separation of a crystalline hemisulfate **3** in 72% yield.

N-2 Methylation of guanine moiety cannot be achieved directly. Therefore, *N*-2-methylacyclovir **4** was synthesized indirectly by using a procedure developed recently for guanosine and deoxyguanosine.[30] When built into a tricyclic 1,N-2-isopropeno derivative, the originally exocyclic NH_2 group at C-2 becomes an endocyclic NH in the resulting imidazole ring and is easily methylated. The *N*-2-methylguanine derivative is then obtained upon removal of the isopropene group with *N*-bromosuccinimide (NBS) followed by alkaline hydrolysis.

Treatment of the 1-sodium derivative of acyclovir in dimethylformamide with bromoacetone followed by concentrated aqueous ammonia gave tricyclic 9-[(2-hydroxyethoxy)methyl]-1,*N*-2-isopropenoguanine[27] (**5**) in 80% yield.

Methylation of **5** with methyl iodide in the presence of potassium carbonate in dimethylformamide resulted in **6** in 77%. Deblocking of **6** with *N*-bromosuccinimide and subsequent alkaline hydrolysis provided **4** in 68% yield.

The tricyclic compounds **5** and **6** can be considered as di-N- and tri-N-substituted derivatives of acyclovir. The 1,*N*-2-isopropeno link is stable under refluxing in water, diluted acid, and diluted base (see the Experimental Section) but can be removed with NBS as described above. The mesoionic compound **7**, another tri-N-substituted analogue of **1a**, was prepared in 92% yield upon methylation of **5** with dimethyl sulfate in dimethylformamide and careful adjustment of pH.

The structure of the novel N-substituted congeners of acyclovir were confirmed and characterized by elemental analyses, proton magnetic resonance spectra (Table I), ultraviolet spectra, and thin-layer chromatography (Table II).

Antiviral, Antimetabolic, and Antitumor Cell Properties

Of the newly synthesized N-substituted derivatives of acyclovir (compounds **2-7**), two compounds (**4** and **6**) proved totally inactive as antiviral agents and two compounds (**3** and **7**) showed anti-HSV activity only at a relatively high concentration (70 μg/mL), whereas the remaining two compounds (**2** and **5**) were inhibitory to HSV-1 and HSV-2 replication at concentrations that were approximately 10-fold higher than those required for acyclovir (**1a**) to inhibit HSV-1 and HSV-2 replication (Table III). Compounds **2** and **5** were also inhibitory to VZV and EHV-1, but only at concentrations that were 30- to 100-fold higher than those found inhibitory for acyclovir. No activity was observed with any of the compounds against VV, VSV, CMV, TK^- HSV-1, or TK^- VZV. As compounds **2** and **5** were most active against HSV-1 and HSV-2, less active against VZV, and not active against CMV, VV, and TK^- variants of HSV-1 and VZV, their activity spectrum appeared to be remarkably similar to that of acyclovir, which suggests that they act in a similar fashion as acyclovir. The inactivity of **2** and **5** against TK^- HSV-1 and VZV mutants further indicates that, like **1a**, compounds **2** and **5** depend for their antiviral activity on a specific phosphorylation by the virus-encoded thymidine kinase.

None of the N-substituted acyclovir derivatives exhibited an appreciable inhibitory effect on host cell DNA, RNA or protein synthesis (Table III). Nor did they exert a cytostatic effect on a number of tumor cells (L1210, FM3A, Raji, Molt/4F). In contrast with acyclovir, which inhibited host cell DNA synthesis (as monitored by incorporation of [*methyl*-^{3}H]dThd) at a concentration as low as 8 μg/mL, its derivatives **2** and **5** failed to do so at a concentration up to 400 or 250 μg/mL, respectively. On the basis of the ratio of the minimum antiviral concentration (against HSV-1 or HSV-2) to the minimum antimetabolic concentration (against DNA synthesis), the following selectivity indexes could be calculated: >400 for **2**, >250 for **5**, as compared to 80 for acyclovir. Thus, under the experimental conditions used, compounds **2** and **5** appear more selective in their anti-HSV action than acyclovir. As **2** and **5** are quite selective anti-HSV agents and, moreover, achieve their antiviral potency at concentrations (0.7–2 μg/mL) that may be readily achievable in the blood stream, these compounds should be further explored for their potential in the treatment of HSV-1 and HSV-2 infections.

From a structural viewpoint it is clear that methylation at either N-2 (compound **4**) or N-7 (compound **3**) virtually annihilated the antiviral activity of acyclovir. Position N-1 seems to be less critical, because 1-methylacyclovir (**2**) has considerable antiviral activity. Akin to compound **4**, compound **5**, in which the exocyclic NH_2 group at C-2 is blocked, may have been expected as inactive. This did not prove to be the case as **5** exhibited a potent and selective antiherpetic activity.

One might suspect that **5** would spontaneously decompose to acyclovir **1a**, i.e. when preparing the test solutions or incubating them with the cells. Transformation of 1,*N*-2-isopropenoguanine to guanine may be achieved by treatment with *N*-bromosuccinimide.[30] However, in the absence of the latter reagent, the 1,*N*-2-isopropeno linkage is highly stable. Compound **5** remained completely unchanged when refluxed in water, 0.1 N hydrochloric acid, or 0.1 N sodium hydroxide for 1 h.

Most likely, **5** is intrinsically active as an antiviral agent, and this activity probably resides with the NH group at C-2. Incorporation of the originally exocyclic NH_2 at C-2 of guanine as an endocyclic NH in the newly arising imidazole ring may endow it with new properties, i.e. stronger proton delivering potential. Blocking of the exocyclic NH_2

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Table III. Antiviral, Antimetabolic, and Antitumor Cell Effects of Various N-Substituted Derivatives of Acyclovir in Vitro

assay	cells	minimum inhibitory concentration,[a] μg/mL						
		2	3	4	5	6	7	acyclovir (1a)
Antiviral Activity								
HSV-1 (KOS)	PRK	1	70	>400	2	>400	70	0.2
HSV-1 (F)	PRK	2	70	>400	2	>400	70	0.1
HSV-1 (McIntyre)	PRK	2	70	>400	2	>400	70	0.2
HSV-2 (G)	PRK	1	40	300	0.7	>400	70	0.07
HSV-2 (Lyons)	PRK	2	100	>400	2	>400	70	0.2
HSV-2 (196)	PRK	2	100	>400	0.7	>400	70	0.07
VV	PRK	>400	>400	>400	>400	>400	>400	150
TK⁻ HSV-1 (B2006)	PRK	300	>400	>400	400	>400	>400	20
TK⁻ HSV-1 (VMW 1837)	PRK	300	>400	>400	400	>400	>400	20
VZV (YS)	HEL	20	>100	>100	20	>100	>100	0.3
VZV (Oka)	HEL	20	>100	>100	20	>100	>100	0.6
TK⁻ VZV (YSR)	HEL	>100	>100	>100	>100	>100	>100	25
TK⁻ VZV (7-1)	HEL	>100	>100	>100	>100	>100	>100	10
CMV (Davis)	HEL	>100	>100	>100	>100	>100	>100	20
CMV (AD-169)	HEL	>100	>100	>100	>100	>100	>100	25
SHV-1	PRK	70	>400	>400	>400	>400	>400	7
BHV-1	PRK	>400	>400	>400	>400	>400	>400	7
EHV-1	PRK	10	>400	>400	20	>400	>400	0.2
Antimetabolic Activity								
[*methyl*-^{3}H]dThd	PRK	>400	>400	>400	257 ± 6	>400	>400	8 ± 3
Antitumor Cell Activity								
	L1210	>100	>100	>100	>100	>100	>100	55 ± 18
	FM3A	>100	>100	>100	>100	>100	>100	66 ± 29

[a] Required to inhibit virus-induced cytopathogenicity (in PRK cells), virus plaque formation (in HEL cells), DNA, RNA, or protein synthesis in PRK cells (monitored by the incorporation of [*methyl*-^{3}H]dThd, [5-^{3}H]Urd or [4,5-^{3}H]Leu, respectively), or tumor (murine leukemia L1210, murine mammary carcinoma FM3A, human B-lymphoblast Raji, human T-lymphoblast Molt/4F) cell proliferation by 50%. None of the compounds proved inhibitory to VSV-induced cytopathogenicity (in PRK cells), and [5-^{3}H]Urd or [4,5-^{3}H]Leu incorporation (in PRK cells) at a concentration of 400 μg/mL (data not shown). Nor did any of the compounds prove inhibitory to the proliferation of Raji or Molt/4F cells at a concentration of 100 μg/mL (data not shown).

by methylation, as in compound 4, clearly behaves differently from incorporation of this group as an endocyclic NH in an imidazole ring, as in compound 5. This is testified by the proton NMR spectra (Table I) in which the proton of $NHCH_3$ of 4 appears at 6.37 ppm whereas the proton of N-2-H of 5 appears at 12.42 ppm. To result in a significant antiviral activity, the endocyclic NH of compound 5 must be intact, since methylation of this group (compound 6) nullifies the antiviral activity.

Experimental Section

General Methods. Melting points were determined on a Boetius Nagema Rapido micromelting point apparatus and are uncorrected. The ultraviolet spectra were measured on a Zeiss Specord UV–vis and on a Zeiss VSU-2P spectrophotometer. The ^{1}H NMR spectra were recorded on a JEOL FX 90Q FT NMR spectrometer. Thin-layer chromatography was conducted on Merck precoated silica gel F_{254} Type 60 plates with the following solvent systems (measured by volume): A, 2-propanol–concentrated aqueous ammonia–water (7:1:2); B, 1-butanol–glacial acetic acid–water (5:3:2); C, chloroform–methanol (4:1). For a preparative short column chromatography, Merck TLC gel HF_{254} Type 60 was used. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer and a Hewlett-Packard 185 CHN analyzer, and the results are within 0.4% of the theoretical values.

1-Methyl-9-[(2-hydroxyethoxy)methyl]guanine (2). Sodium hydride (13 mg, 0.53 mmol) in 50% suspension in oil was added to a suspension of acyclovir (1a; 112.6 mg, 0.5 mmol) in anhydrous dimethylformamide (3 mL), and this mixture was stirred with exclusion of moisture for 10 min. The resulting clear solution was treated with methyl iodide (75 mg, 0.53 mmol) and then maintained at room temperature for 25 min. After this time the reaction mixture was evaporated to dryness, and an oily residue coevaporated with water. The obtained white solid was crystallized from water: yield 67.2 mg (56%); mp 253 °C dec. Anal. ($C_9H_{13}N_5O_3 \cdot 0.25H_2O$) C, H, N.

7-Methyl-9-[(2-hydroxyethoxy)methyl]guanine Hemisulfate (3). A suspension of acyclovir (1a; 225 mg, 1.0 mmol) in dimethylformamide (3 mL) was treated with dimethyl sulfate (164 mg, 1.3 mmol) and stirred at room temperature for 20 h. The resulting clear solution was cooled to 0 °C, adjusted to pH 8.5 with concentrated aqueous ammonia, and then diluted with acetone (20 mL). The white precipitate that appeared was separated by filtration, washed with acetone and ethyl ether, and dried in vacuo. The crude product was crystallized from 50% aqueous methanol to give crystalline 3 (206 mg, 72%), mp 231 °C dec. Anal. ($C_9H_{13}N_5O_3 \cdot 0.5H_2SO_4$) C, H, N; S: calcd, 5.56; found, 6.15.

9-[(2-Hydroxyethoxy)methyl]-1,*N*-2-isopropenoguanine (5). To an anhydrous suspension of 1a (676 mg, 3.0 mmol) in dimethylformamide (18 mL) was added sodium hydride (76 mg, 3.18 mmol) in 50% suspension in oil. After being stirred with exclusion of moisture for 15 min, the solution thus obtained was treated with bromoacetone (436 mg, 3.18 mmol). The reaction mixture was stirred for the next 30 min, made alkaline by addition of concentrated aqueous ammonia (20 mL), and left overnight at room temperature. The dark red solution was evaporated to dryness, and the resulting oil was coevaporated first with water and then with ethanol. The residue was dissolved in chloroform–methanol (6:1, 8 mL), applied onto a silica gel short column (3.5 × 9 cm) and chromatographed in this solvent system. Fifteen-milliliter fractions were collected. Fractions 27–51 containing the main product were pooled and evaporated to dryness to give the crude product 5 as a white solid (630 mg, 80%), homogeneous by TLC, mp 207–210 °C dec. An analytical sample was crystallized from ethyl acetate–methanol (5:1), mp 219 °C dec. Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

Stability of Compound 5. Three samples of 5 (10 mg each) were refluxed in either water, 0.1 N hydrochloric acid, or 0.1 N sodium hydroxide (1.5 mL each) for 1 h. After being cooled and neutralized, the samples were analyzed by thin-layer chromatography. In all three experiments compound 5 appeared to be completely stable; in particular, traces of acyclovir (1a) could not be detected.

***N*-2-Methyl-9-[(2-hydroxyethoxy)methyl]-1,*N*-2-isopropenoguanine (6).** To a solution of 5 (263 mg, 1.0 mmol) in dimethylformamide (5 mL) was added a portion of well-powdered potassium carbonate (207 mg, 1.5 mmol), and after the mixture was stirred for 30 min, methyl iodide (170 mg, 1.2 mmol) was added as well. The methylation reaction was carried out for 45 min and then quenched by addition of water (2 mL), and the

solution was evaporated to dryness. The residue was dissolved in ethanol (15 mL) and adsorbed on a portion of silica gel (5 g, 50–100 mesh) by evaporation. The dried gel was applied onto a silica gel short column (3.5 × 8 cm). Elution was performed with chloroform–methanol (6:1); 12-mL fractions were collected. Fractions 13–29 contained chromatographically pure product **6** as a white powder (212 mg, 77%) after evaporation and drying. An analytical sample was crystallized from ethanol, which gave fine crystals of **6**, mp 256 °C dec. Anal. ($C_{12}H_{15}N_5O_3$) C, H, N.

***N*-2-Methyl-9-[(2-hydroxyethoxy)methyl]guanine (4).** To a solution of **6** (138.6 mg, 0.50 mmol) in water (10 mL) was added *N*-bromosuccinimide (98 mg, 0.55 mmol) with stirring. After 25 min of being stirred, the reaction mixture was alkalized with concentrated aqueous ammonia (15 mL) and left at room temperature for 30 min. The solution was concentrated to a volume of 15 mL, diluted with ethanol (20 mL), and evaporated with a portion of silica gel (3 g, 50–100 mesh). The dried gel was applied onto a silica gel short column (2.5 × 5 cm). Elution was performed with solvent C (see above), and 10-mL fractions were obtained. Fractions 12–28 containing the main product were concentrated to a volume of ca. 100 mL and left at 5 °C for 2 days. The resulting crystalline material was collected by filtration, washed with ether, and dried in vacuo to give 82.1 mg of **7** (68%), mp 232–237 °C (dec without melting point). Anal. ($C_9H_{13}N_5O_3 \cdot 1.5H_2O$) C, H; N: calcd, 26.30; found, 25.69.

7-Methyl-9-[(2-hydroxyethoxy)methyl]-1,*N*-2-isopropenoguanine (7). A solution of **5** (105.3 mg, 0.40 mmol) in dimethylformamide (2 mL) was treated with dimethyl sulfate (50 mg, 0.48 mmol). After it had been left for 30 h at room temperature, the reaction mixture was diluted with acetone (5 mL) and ethyl ether (2 mL), cooled to −5 °C, and adjusted to pH 9.5 with concentrated aqueous ammonia. The resulting white precipitate was immediately collected by filtration on a suction funnel, washed with acetone and ethyl ether, and dried under diminished pressure to give **7** as a white powder (102.3 mg, 92%) homogeneous by TLC, mp 139 °C. Anal. ($C_{12}H_{15}N_5O_3$) H, N; C: calcd, 51.98; found, 52.46.

Biological Activity Evaluation. The assays used for measuring antiviral activity (based on inhibition of either virus-induced cytopathogenicity or plaque formation), antimetabolic activity (based on inhibition of DNA, RNA, and protein synthesis), and antitumor cell activity (based on inhibition of tumor cell proliferation) have been described previously.[32,33]

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cis-4-Carboxy-6-(mercaptomethyl)-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one, a Potent Inhibitor of Mammalian Dihydroorotase

Jerry L. Adams,*[1a] Thomas D. Meek,[1a] Shau-Ming Mong,[1b] Randall K. Johnson,[1b] and Brian W. Metcalf[1a]

Departments of Medicinal Chemistry and Molecular Pharmacology, Research and Development Division, Smith Kline & French Laboratories, 709 Swedeland Road, Swedeland, Pennsylvania 19479. *Received December 7, 1987*

A series of *cis*- and *trans*-4-carboxy-3,4,5,6-tetrahydropyrimidin-2(1*H*)-ones possessing either a carboxy, hydroxymethyl, or mercaptomethyl substituent at C-6 were prepared and tested for their ability to inhibit mammalian dihydroorotase. Of these compounds, only the *cis*-6-mercaptomethyl compound, *cis*-**1**, was found to be a potent competitive inhibitor of the enzyme (K_i = 140 nM at pH 7.4 and 8.5) when assayed in the direction of dihydro-L-orotate hydrolysis. These results suggest that the inhibition arises from the ligation of the thiolate to the zinc atom which is thought to be located in the enzyme's active site.[2,3] Although analysis of *cis*-**1** with 2,2′-dithiobis(5-nitrobenzoic acid) revealed significant loss of the free thiol group under enzymatic assay conditions, the addition of the reducing agent, dithiothreitol, to the enzymatic reaction mixtures afforded *cis*-**1** complete protection against this chemical decomposition, as evidenced by lowering of the inhibition constant in the presence of dithiothreitol. Compound *cis*-**1** had no significant antiproliferative activity against B16 melanoma cells in tissue culture, possibly due to the rapid decomposition of the compound or poor permeability into cells.

The mammalian CAD protein is a trifunctional protein containing the first three enzymes of the de novo pyrimidine nucleotide biosynthetic pathway: carbamoylphosphate synthetase II, aspartate transcarbamoylase (ATCase), and dihydroorotase. The elevation in neoplastic tissues of the enzymatic activities of this complex as well as other key enzymes involved in the biosynthesis of DNA has been noted as an important consequence of the biochemical commitment of cancer cells to replicate.[4] This observation suggests that inhibition of these key enzymes may be a valid approach for the design of new antineoplastic agents.

N-(Phosphonoacetyl)-L-aspartate (PALA) is an extremely potent inhibitor of ATCase and is highly effective in a number of experimental tumor models.[5,6] However,

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