Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 1247–1254
Bioorganic & Medicinal Chemistry

Synthesis, antiviral, and antitumor activity of 2-substituted purine methylenecyclopropane analogues of nucleosides

Xinrong Qin,[a] Xinchao Chen,[a] Kun Wang,[a] Lisa Polin,[a] Earl R. Kern,[b] John C. Drach,[c] Elizabeth Gullen,[d] Yung-Chi Cheng[d] and Jiri Zemlicka[a,*]

[a]*Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201-1379, USA*
[b]*Department of Pediatrics, The University of Alabama School of Medicine, Birmingham, AL 35233, USA*
[c]*Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48019-1078, USA*
[d]*Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510-8066, USA*

Received 28 July 2005; revised 20 September 2005; accepted 22 September 2005
Available online 18 October 2005

Abstract—The *Z*- and *E*-2-fluoro- and 2-chloropurine methylenecyclopropanes **9a**,**b** and **10a**,**b** as well as enantiomeric *Z*-isoguanine methylenecyclopropanes **11a**,**b** and their phenyl phosphoralaninate pronucleotides **11c**,**d** were synthesized and their antiviral activity against several viruses was evaluated. Fluoro analogues **9a** and **10a** were active against human cytomegalovirus but they were cytotoxic at approximately the same concentrations. Chloro derivatives **9b** and **10b** were non-cytotoxic and effective against Epstein–Barr virus in Daudi cells. Isoguanine analogues **11a**–**d** lacked antiviral activity but pronucleotides **11c**,**d** were substrates for porcine liver esterase. From the group of **9a**,**b** and **10a**,**b**, the fluoro analogues **9a** and **10a** exhibited antitumor activity but only the *Z*-isomer **9a** had a selective effect.

1. Introduction

Methylenecyclopropane analogues of nucleosides are a new class of antiviral agents. Their antiviral activity is primarily associated with the purine *Z*-isomers **1** but *E*-isomers **2** (Chart 1) are also effective in some cases.[1,2] The most prominent antiviral effects of these analogues include inhibition of the human and murine cytomegalovirus (HCMV and MCMV) as well as Epstein–Barr virus (EBV). The activity of these analogues can be broadened and increased by conversion to phenyl phosphoralaninate prodrugs **1b** and **2b**. For example, transformation of compounds **3a** and **4a** (enantiomeric or racemic) to pronucleotides **3b** and **4b** led to potent agents effective against HIV and hepatitis B virus (HBV).[3,4] Extensive structure–activity relationship (SAR) studies have indicated[5] that antiviral potency of 2-aminopurine methylenecyclopropanes **5** is compatible with a wide range of N-, O-, and S-substituents in the

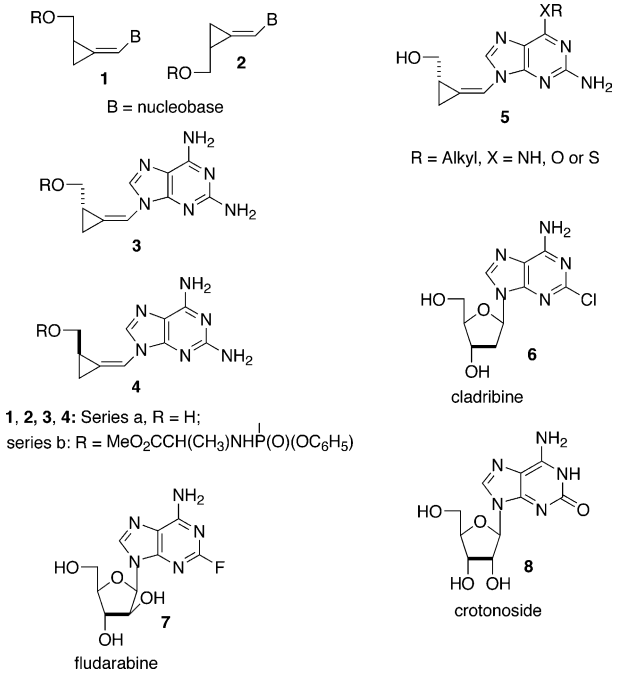


Chart 1.

Keywords: Methylenecyclopropanes; Nucleoside analogues; Pronucleotides; Antiviral and antitumor agents; Porcine liver esterase.
* Corresponding author. Tel.: +1 313 833 0715; fax: +1 313 832 7294; e-mail: zemlicka@kci.wayne.edu

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.09.052

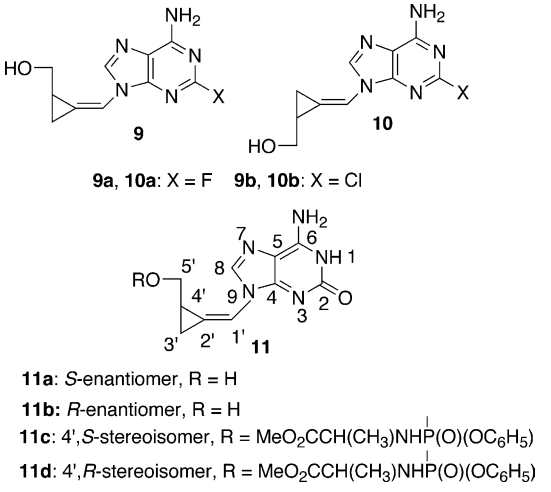
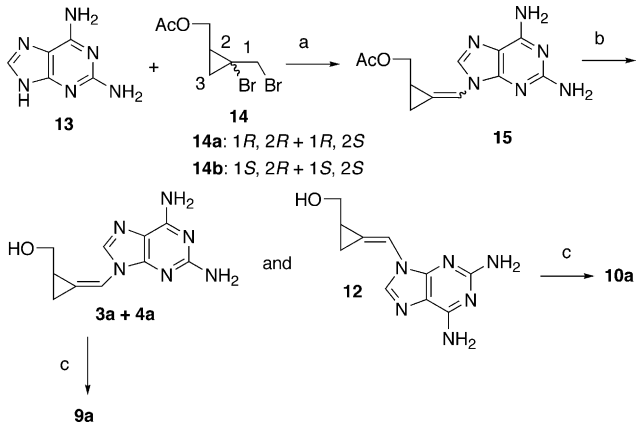


Chart 2.



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, Δ; (b) 1—K_2CO_3, MeOH/H_2O; 2—chromatography; (c) *t*-BuONO, 70% HF–pyridine, pyridine–toluene, −30 °C.

position 6 of the purine ring. By contrast, effects of replacement of the 2-amino function with other groups have not been investigated. In the nucleoside series, 2-chloro-2′-deoxyadenosine (**6**, cladribine) and 9-(β-D-arabinofuranosyl)-2-fluoroadenine (**7**, fludarabine) are antileukemic agents.[6–8] Another nucleoside analogue, isoguanosine (**8**), which has an oxo group in the purine 2-position, is a natural product (crotonoside)[9,10] not found in RNA. It is not associated with any substantial biological activity but the triphosphate of the 2′-deoxyisoguanosine is a substrate[11] for reverse transcriptase of HIV-1.

It was then of interest to investigate the synthesis and biological activity of 2-substituted purine methylenecyclopropane analogues **9a**,**b**, **10**,**b**, and **11a**,**b** and phosphoramidates **11c**,**d**. 2-Fluoro- and 2-chloropurines **9a**,**b** and **10a**,**b** are racemic *Z*- and *E*-isomers, whereas isoguanine analogues **11a**,**b** are enantiomeric *Z*-isomers. Analogues **11c**,**d** are then phenyl phosphoralaninates of **11a**,**b** related to antiviral pronucleotides[3,4] **3b** and **4b** (Charts 1 and 2).

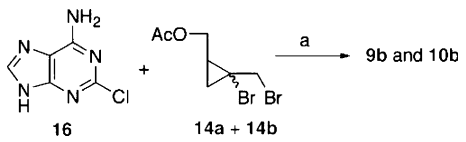
2. Results and discussion

2.1. Synthesis

2-Aminosynadenol (**3a**, **4a**, or **3a** + **4a**) and the *E*-isomer **12** (Scheme 1) were attractive starting materials for synthesis of the desired target compounds, racemic or enantiomeric by diazotization/halogenation procedure[12] (**9a**,**b** and **10a**,**b**) or by hydrolytic diazotization[13] (**11a**,**b**). Compounds **3a**, **4a**, **3a** + **4a**, and (±)-**12** were previously obtained using 2-amino-6-chloropurine as a starting material.[4,14] Because 2,6-diaminopurine (**13**) is significantly less expensive than 2-amino-6-chloropurine, it was used in an alkylation–elimination procedure[15] with dibromocyclopropanes[16] **14a**,**b** or **14a** + **b** as shown for racemic compound **14a** + **b** in Scheme 1. The intermediary *Z*,*E*-acetate (±)-**15** obtained in 45% yield was deprotected to give, after chromatographic separation, the *Z*- and *E*-isomers **3a** + **4a** and (±)-**12** (47% and 33% yield, respectively). A similar procedure was recently used for synthesis of 2,3-bis-(hydroxymethyl)methylenecyclopropane nucleoside analogues.[17]

The reaction of 2-aminosynadenol (**3a** + **4a**) with *tert*-butyl nitrite (TBN) and HF–pyridine[12] in toluene at −30 °C gave 2-fluorosynadenol (**9a**) in 15% yield (Scheme 1). In a similar fashion, the *E*-isomer (±)-**12** afforded the 2-fluorinated analogue **10a** (21%). Because TBN/$SbCl_3$, a reagent employed previously for diazotization–chlorination of 2-aminopurine nucleosides[12] did not give any product with 2-aminosynadenol (**3a** + **4a**), another approach was adopted. 2-Chloro-6-aminopurine (**16**) was subjected to alkylation–elimination procedure with reagent **14a** + **b** to give, after deacetylation, a mixture of the *Z*- and *E*-isomers **9b** and **10b** (*Z*/*E* = 1.2/1) in 12% yield (Scheme 2). Attempted separation of isomers on silica gel was unsuccessful but a smooth resolution was achieved on aluminum oxide column. The *Z*/*E* isomeric assignment followed from the NMR spectra (Table 1). The relevant signals of **9b** and **10b** follow the same pattern as synadenol and its *E*-isomer[18] or fluoro analogues **9a** and **10a**, which were prepared separately from the *Z*- and *E*-isomers **3a** + **4a** and (±)-**11**. Thus, the H_8 and $C_{4'}$ of the *Z*-isomers **9a**,**b** are more deshielded than those of the *E*-isomers **10b**,**b**, whereas the opposite is true for the $H_{1'}$ and $C_{3'}$. A typical non-equivalency[18] of the $H_{5'}$ protons of the *Z*-isomers **9a**,**b** is also preserved.

Hydrolytic diazotization of enantiomers **3a** and **4a** using TBN or $NaNO_2$ in 80% AcOH gave the corresponding isoguanine analogues **11a**,**b** in 37–53% yield (Scheme 3). Attempted reaction of the (*S*,*Z*)-enantiomer **3a** with phosphorylating agent **17** gave only 2-*O*-phosphorylated



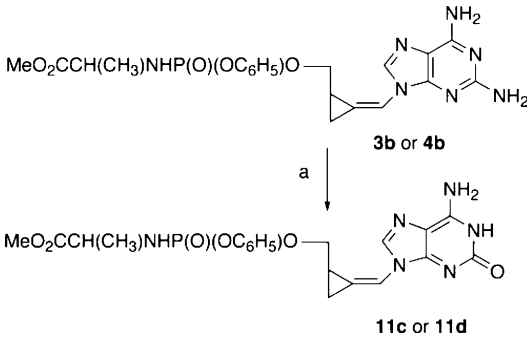
Scheme 2. Reagents and conditions: (a) 1—K_2CO_3, DMF, Δ; 2—H_2O; 3—chromatography.

Table 1. Selected ^{1}H and ^{13}C NMR chemical shifts[a] of analogues **9a**,**b** and **10a**,**b**

Compound	H_8	$H_{1'}$	$H_{5'}$	$C_{3'}$	$C_{4'}$
9a	8.67	7.24	3.28, 3.68	7.0	20.0
10a	8.44	7.36	3.40	10.0	18.4
9b	8.71	7.29	3.32, 3.70	7.1	20.0
10b	8.48	7.40	3.40	9.9	18.4

[a] For numbering of atoms, see formula **11** in Chart 2. ^{1}H NMR chemical shifts are in δ units.

product **18** derived from a lactim form of **11a** in 27% yield. The structure was supported by a similarity of UV spectrum to those of other 2-*O*-substituted isoguanosines[19] and the presence of hydroxy group signal in the ^{1}H NMR spectrum. It was reported that strong electrophiles (benzoylation or tosylation)[19] attack the lactim form of isoguanosine. Because pronucleotides such as **3b** or **4b** are stable in acid,[3,4] a hydrolytic diazotization with TBN in 80% AcOH was attempted. Indeed, compounds **3b** and **4b** gave the desired pronucleotides **11c** and **d** in 50% and 68% yield, respectively (Scheme 4).



Scheme 4. Reagents: (a) *t*-BuONO, 80% AcOH.

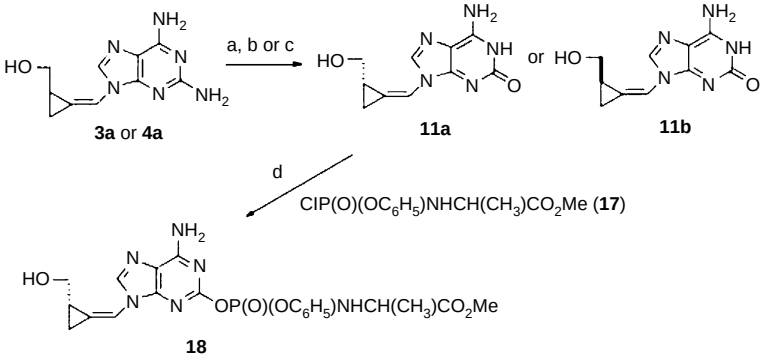
2.2. Antiviral activity

The antiviral data are summarized in Table 2. None of the analogues was effective against HSV-1, HSV-2, HIV-1, or HBV (data not presented). The fluoro analogues **9a** and **10a** inhibited the replication of HCMV in Towne and AD169 strains of the virus but the antiviral effects were poorly separated from cytotoxicity. Both compounds were inactive against EBV in Daudi and H-1 cells but, again, cytotoxicity was apparent in the latter culture. By contrast, chloro analogues **9b** and **10b** were non-cytotoxic and a potent antiviral effect was seen in EBV-infected Daudi cells by viral capsid antigen (VCA) ELISA. Both analogues had $EC_{50} < 0.08$ μM. These results indicate that chloro analogues **9b** and **10b** are, in principle, capable of intracellular phosphorylation to the triphosphate level. Nevertheless, little activity against EBV was detected in the H-1 culture by DNA hybridization assay. Similar differences between both types of assays were observed before in some other methylenecyclopropane analogues.[4,5] The 2,6-diaminopurine analogues **3a** and **4a** are effective agents against EBV[14] and moderately potent against HBV and HCMV but isoguanine counterparts **11a**,**b** were devoid of antiviral activity (Table 2). In contrast to pronucleotides **3b** and **4b** which exhibit potent antiviral activity,[4] phosphoralaninates **11c**,**d** were without significant effect (Table 2).

Interestingly, both active and inactive phosphoralaninates were substrates (Table 3) for porcine liver esterase (PLE) which is considered as a good model of intracellular esterases.[20,21] According to a general pattern, pronucleotides are converted to phosphoralanines **19** as indicated in Scheme 5. Pronucleotides **4b** and **11d** derived from the 4′,*R*-configured methylenecyclopropanes **4a** and **11b** were hydrolyzed noticeably faster than **3b** and **11c** which correspond to the 4′,*S*-configured parent compounds **3a** and **11a**. At any rate, taken together these results have indicated that factors other than intracellular hydrolysis of alanine ester moiety must be responsible for inactivity of phosphoramidates **10c**,**d**.

2.3. Antitumor activity

Analogues **9a**,**b** and **10a**,**b** were investigated in several tumor systems in vitro by disk-diffusion assay[22] (Table 4). The antitumor effect of the 2-fluoropurine *Z*-isomer **9a** indicated some selectivity in comparison with normal fibroblast cells. There was little differentiation of activity against solid tumors versus leukemia L1210. The *E*-isomer **10a** was cytotoxic across the board. Chloro analogues **9b** and **10b** were inactive.



Scheme 3. Reagents: (a) *t*-BuONO, 80% AcOH; (b) $NaNo_2$, 80%AcOH, 1 M HCl; (c) $NaNO_2$, 80% AcOH; (d) 1-methylimidazole, pyridine.

Table 2. Inhibition of HCMV, EBV, and VZV replication by 2-fluoro-, 2-chloro-, and 2-oxopurine methylenecyclopropane analogues **9a**, **10a**, **9b**, **10b**, and **11a–d**

Compound	EC_{50}/CC_{50} (μM)				
	HCMV/HFF		EBV		VZV/HFF[c,g]
	Towne[a,b]	AD169[c,d]	Daudi[e]	H-1[f]	
9a	31/10	>4/12.8	>50/>50	>20/8.5	>4
10a	3.5/10	>0.8/1.5	>50/>50	>20/1.4	>0.8
9b	>100/>100	>60/>300	<0.08/>50	>20/>100	>60
10b	>100/>100	231/>300	<0.08/>50	>20/>100	179
11a	>100/>100	>85.8/>429	>214/>214	>20/61	>85.8
10b	>100/>100	>85.8/>429	>214/>214	>20/86	>429
11c	>100/>100	>40.9/>205	55.4/>102	>20/>100	>205
11d	>100/>100	>208/>208	70.4/>104	>20/69	>41.7
Control	1.7/>100[h]	0.22/40[h]	0. 93[i]	5[h]	0.22[i]

[a] Plaque reduction assay.
[b] Visual cytotoxicity in stationary cells.
[c] Cytopathic effect (CPE) inhibition assay.
[d] Cytotoxicity by neutral red uptake.
[e] Viral capsid antigen (VCA) ELISA.
[f] DNA hybridization assay. Cytotoxicity was determined in CEM cells.
[g] For cytotoxicity see HCMV(AD169)/HFF.
[h] Ganciclovir.
[i] Acyclovir.

Table 3. Hydrolysis of pronucleotides **3b**, **4b**, and **11c**,**d** with PLE at pH 7.4 and room temperature[a]

Pronucleotide	Reaction time, h (>90% hydrolysis)
3a (Ref. 4)	7
4a (Ref. 4)	0.25
11c	6
11d	1

[a] For details, see Section 4.

3. Conclusion

2-Fluoro-, 2-chloro, and 2-oxopurine methylenecyclopropane nucleoside analogues **9a**, **10a**, **9b**, **10b**, and **11a**,**b** were synthesized and evaluated for antiviral activity. The pronucleotides **11c**,**d** were also obtained. Fluoro analogues **9a** and **10a** were moderately effective against HCMV. Their cytotoxicity was apparent throughout the spectrum of antiviral assays. Chloro analogues **9b** and **10b** were non-cytotoxic and they were very effective against EBV in Daudi cells but inactive in H-1 culture. 2-Oxopurines **11a**,**b** and pronucleotides **11c**,**d** were inactive. Compounds **11c**,**d** were as effective substrates for porcine liver esterase as potent antivirals **3b** and **4b**. Fluoro analogues **9a** and **10a** are antitumor agents with limited selectivity.

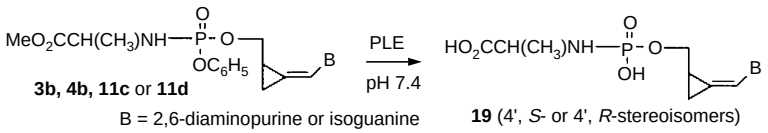
4. Experimental

4.1. General methods

The UV spectra were measured in ethanol. The NMR spectra were recorded at 400 (^{1}H), 100 (^{13}C), and 376 (^{19}F) MHz in CD_3SOCD_3. For ^{19}F NMR, $CFCl_3$ was used as a reference. Mass spectra were determined in an electron-impact (EI-MS) or electrospray ionization (ESI) mode using MeOH and NaCl.

4.2. Starting materials

2,6-Dichloropurine was either a commercial product or it was prepared as described.[23] 6-Amino-2-chloropurine (**16**) was synthesized from 2,6-dichloropurine.[24]



Scheme 5.

Table 4. Antitumor activity of fluoro analogues **9a** and **10a** in a disk diffusion assay[a]

Compound	μg/disk	Leukemia L1210	Mouse P03[b]	Human HCT15/MDR[c]	Normal cells (fibroblasts)
9a	100	400–650	600	400–600	0–200
10a	100	700	700	400–700	600
SR271425[28]	11	0–190	700–800	60–150	0–110

[a] For details, see Section 4.14.
[b] Mouse pancreatic adenocarcinoma 03.
[c] Multiple drug resistant colon tumor.[27]

4.3. (±)-*Z*- and *E*-2,6-Diaminopurine-9-[2-(hydroxymethyl)cyclopropylidene]methylpurine (3a + 4a) and (±)-12

A mixture of 2,6-diaminopurine (**13**, 6.98 g, 0.026 mol), dibromoester **14a** + **b** (5 g, 0.0175 mol), and K_2CO_3 (19.3 g, 0.14 mol) was stirred in DMF (100 mL) under N_2 at 100 °C for 24 h. The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column using CH_2Cl_2–MeOH (9:1) to give the (*Z*,*E*)-acetates (±)-**15** (2.2 g, 45%). A mixture of (±)-**15** (1.8 g, 6.9 mmol) and K_2CO_3 (0.96 g, 6.9 mmol) in 90% MeOH (620 mL) was stirred at room temperature for 3 h. The solvent was evaporated and the crude product was chromatographed as described above to afford the *Z*-isomer **3a** + **4a** (750 g, 47%) and *E*-isomer (±)-**12** (550 mg, 33%) which were identical with the products prepared by another route.[14]

Enantiomeric *Z*-isomers **3a** and **4a** were prepared as described above from dibromoesters[16] **14a** or **b** using Cs_2CO_3 instead of K_2CO_3 (reaction time 40 h at 80 °C) to give (*R*,*Z*,*E*)- or (*S*,*Z*,*E*)-acetates **15** (55% yield). Deacetylation was performed as described above in 20% aqueous methanol, and the *Z*- and *E*-isomers were separated by chromatography using 6–10% MeOH in CH_2Cl_2. (*S*,*Z*)-Isomer **3a**: mp 238–240 °C, lit.[4] 235–238 °C; $[\alpha]_D^{20}$ 84.8 (*c* 0.33, DMF), lit.[4] $[\alpha]_D^{25}$ 78.0 (*c* 0.32, DMF). Isomer (*S*,*E*)-**12**: mp 199–201 °C, lit.[14] 207–210 °C (racemate); $[\alpha]_D^{20}$ 28.6 (*c* 0.21, DMF). (*R*,*Z*)-Isomer **4a**: mp 239–241 °C, lit.[4] 236–239 °C; $[\alpha]_D^{20}$ −79.1 (*c* 0.23, DMF), lit.[4] $[\alpha]_D^{20}$ −81.2 (*c* 0.31, DMF). Isomer (*R*,*E*)-**12**: mp 197–199 °C, lit.[14] 207–210 °C (racemate); $[\alpha]_D^{20}$ −31.4 (*c* 0.35, DMF). The UV, ^{1}H, and ^{13}C NMR spectra corresponded to the racemic compounds.[14]

4.4. (±)-(*Z*)-2-Fluoro-9-[(2-hydroxymethyl)cyclopropylidene]methyladenine (9a)

HF (70%) in pyridine (2.8 mL) was added to a suspension of compound **3a** + **4a** (276 mg, 1.19 mmol) in a mixture of *tert*-butyl nitrite (TBN, 0.4 mL, 3.0 mmol), pyridine (0.5 mL), and toluene (10 mL) at −30 °C with stirring. The temperature was allowed to rise to room temperature, NEt_3 (2 mL) was added and the volatile components were evaporated in vacuo. The residue was chromatographed on a silica gel column in CH_2Cl_2–MeOH (20:1) to give compound **9a** (41 mg, 14.5%), mp 273–275 °C. UV max 261 nm (ε 13,000), 227 (ε 23,700). ^{1}H NMR δ 1.20 (ddd, 1H, *J* = 8.5, 5.3, 2.0 Hz), 1.45 (td, 1H, *J* = 8.3, 1.5 Hz, $H_{3'}$), 2.09 (m, 1H, $H_{4'}$), 3.28 (m, partly overlapped with H_2O, 1H), 3.68 (dt, *J* = 10.5 and 5.0 Hz, $H_{5'}$), 5.06 (dd, 1H, *J* = 6.0 and 4.5 Hz, OH), 7.24 (d, 1H, *J* = 1.2 Hz, $H_{1'}$), 7.85 (br s, 2H, NH_2), 8.67 (s, 1H, H_8). ^{13}C NMR 7.0 ($C_{3'}$), 20.0 ($C_{4'}$), 63.5 ($C_{5'}$), 110.7 ($C_{1'}$), 117.0 and 117.5 ($C_{2'}$, C_5), 138.9 (C_8), 149.8 (d, *J* = 20 Hz, C_4), 158.3 (d, *J* = 21.1 Hz, C_6), 159.5 (d, *J* = 209.5 Hz, C_2). ^{19}F NMR −52.23. EI-MS 235 (M, 25.0), 218 (M−OH, 60.1), 153 (2-fluoroadenine, 100.0). HRMS calcd for $C_{10}H_{10}FN_5O$: 235.0869. Found: 235.0867. Anal. Calcd for $C_{10}H_{10}FN_5O$: C, 51.06; H, 4.29; N, 29.77. Found: C, 50.90; H, 4.21; N, 29.69.

4.5. (±)-(*E*)-2-Fluoro-9-[(2-hydroxymethyl)cyclopropylidene]methyladenine (10a)

The procedure for the *Z*-isomer **3a** + **4a** was followed with compound (±)-**12** (129 mg, 0.56 mmol). Chromatography afforded the *E*-isomer **10a** (27 mg, 20.5%), mp 263–265 °C. UV max 262 nm (ε 13,100), 227 (ε 25,500). ^{1}H NMR δ 1.37 (m, 1H), 1.69 (td, 1H, *J* = 9.0, 2.5 Hz, $H_{3'}$), 1.96 (m, 1H, $H_{4'}$), 3.40 (t, 2H, *J* = 6.4 Hz, $H_{5'}$), 4.83 (t, 1H, *J* = 6.4 Hz, OH), 7.36 (d, *J* = 1.6 Hz, $H_{1'}$), 7.93, 7.86 (2 partly overlapped br s, 2H, NH_2), 8.44 (s, 1H, H_8). ^{13}C NMR 10.0 ($C_{3'}$), 18.4 ($C_{4'}$), 63.7 ($C_{5'}$), 110.9 ($C_{1'}$), 117.4, 117.5 ($C_{2'}$, C_5), 138.4 (C_8), 150.2 (d, *J* = 20.1 Hz, C_4), 158.3 (d, *J* = 21.6 Hz, C_6), 159.6 (d, *J* = 204.4 Hz, C_2). ^{19}F NMR −52.11. EI-MS 235 (M, 14.5), 218 (M−OH, 100.0), 153 (2-fluoroadenine, 25.5). HRMS calcd for $C_{10}H_{10}FN_5O$ 235.0869. Found: 235.0872. Anal. Calcd for $C_{10}H_{10}FN_5O{\cdot}0.5H_2O$: C, 49.18; H, 4.54; N, 28.68. Found: C, 48.85; H, 4.47; N, 28.62.

4.6. (±)-(*Z*)-6-Amino-2-chloro-9-[2-(hydroxymethyl)cyclopropylidene]methylpurine (9b) and (±)-(*E*)-6-amino-2-chloro-9-[2-(hydroxymethyl)cyclopropylidene]methylpurine (10b)

A mixture of 6-amino-2-chloropurine (**16**, 170 mg, 1.0 mmol), dibromocyclopropane (**14a** + **14b**, 428 mg, 1.5 mmol), and Cs_2CO_3 (2.93 g, 9.0 mmol) was stirred at 70–80 °C in DMF (20 mL) for 40 h under N_2. After cooling, the reaction mixture was filtered and the solid residue was washed with methanol (2 × 20 mL).[25] The filtrate was concentrated in vacuo and the residue was chromatographed on a silica gel column in CH_2Cl_2–MeOH (20:1) to give the (*Z*,*E*) isomeric mixture **9b** and **10b** (31 mg, 12.3%, *Z*/*E* = 1.2:1). This product (100 mg) was chromatographed on an aluminum oxide column in EtOAc–MeOH (18:1) to give the *E*-isomer **9b** (30.5 mg, 30.5%) and *Z*-isomer **10b** (35.2 mg, 35.2%).

E-Isomer **10b**: mp 245–246 °C. UV max 267 nm (ε 14,300), 216 nm (ε 28,900). ^{1}H NMR δ 1.37 (ddd, 1H, *J* = 9.2, 5.5, 2.4 Hz), 1.69 (td, 1H, *J* = 9.2, 2.4 Hz, $H_{3'}$), 1.96 (m, 1H, $H_{4'}$), 3.40 (t, 2H, *J* = 6.4 Hz, $H_{5'}$), 4.81 (t, 1H, *J* = 6.4 Hz, OH), 7.40 (d, 1H, *J* = 2.4 Hz, $H_{1'}$), 7.90 (br s, 2H, NH_2), 8.48 (s, 1H, H_8). ^{13}C NMR 9.9 ($C_{3'}$), 18.4 ($C_{4'}$), 63.8 ($C_{5'}$), 110.9 ($C_{1'}$), 117.7, 118.2 ($C_{2'}$, C_5), 138.0 (C_8), 149.9 (C_4), 154.1 (C_2), 157.5 (C_6). EI-MS 252, 254 (M+H, 39.2, 15.0), 251, 253 (M, 9.3, 6.2), 234, 236 (M−OH, 100.0, 33.5), 170, 172 (2-chloroadenine + H, 39.2, 15.0), 169, 171 (2-chloroadenine, 31.7, 15.4). HRMS calcd for $C_{10}H_{10}{}^{35}ClN_5O$: 251.0574. Found: 251.0575. Anal. Calcd for $C_{10}H_{10}ClN_5O$: C, 47.72; H, 4.00; N, 27.83. Found: C, 47.80; H, 4.17; N, 27.62.

Z-Isomer **9b**: mp 242–243 °C. UV max 266 nm (ε 12,900), 227 (ε 26,700). ^{1}H NMR δ 1.21 (td, 1H, *J* = 8.5, 5.6 Hz), 1.49 (t, 1H, *J* = 8.8 Hz, $H_{3'}$), 2.13 (m, 1H, $H_{4'}$), 3.32 (m, 1H), 3.70 (dt, 1H, *J* = 10.5, 5.0 Hz, $H_{5'}$), 5.06 (t, 1H, *J* = 4.8 Hz, OH), 7.29 (d, 1H, *J* = 1.6 Hz, $H_{1'}$), 7.86 (br s, 2H, NH_2), 8.71 (s, 1H, H_8). ^{13}C NMR 7.1 ($C_{3'}$), 20.0 ($C_{4'}$), 63.5 ($C_{5'}$), 110.8

($C_{1'}$), 117.3, 118.2 ($C_{2'}$, C_5), 139.1 (C_8), 149.7 (C_4), 154.1 (C_2), 157.4 (C_6). EI-MS 251, 253 (M, 17.6, 6.4), 234, 236 (M−OH, 61.8, 20.9), 170, 172 (2-chloroadenine + H, 71.9, 25.4), 169, 171 (2-chloroadenine, 100.0, 40.2). HRMS calcd for $C_{10}H_{10}{}^{35}ClN_5O$: 251.0574. Found: 251.0581. Anal. Calcd for $C_{10}H_{10}ClN_5O$: C, 47.72; H, 4.00; N, 27.83. Found: C, 47.51; H, 4.18; N, 27.60.

4.7. (*S*,*Z*)-9-[2-(Hydroxymethyl)cyclopropylidene]methylisoguanine (11a)

4.7.1. Method A. Using *tert*-butyl nitrite (TBN). TBN (225 mg, 2.16 mmol) was added to a solution of compound **3a** (100 mg, 0.43 mmol) in 80% AcOH (6 mL) at 0 °C in the dark with stirring. The stirring was continued at room temperature for another 10 h. The solvent was removed in vacuo at room temperature and the residue was chromatographed on a silica gel column using CH_2Cl_2–MeOH (4:1) to give recovered starting material **3a** (22 mg, 22%) and compound **11a** (53 mg, 53%). Mp >240 °C (decomp.), $[\alpha]_D^{20}$ 63.1 (*c* 0.32, water). UV max (EtOH) 300 nm (ε 14,000), 232 (ε 41,400). ^{1}H NMR δ 1.17 (poorly resolved t, 1H), 1.45 (t, 1H, *J* = 8.0 Hz, $H_{3'}$), 2.08 (poorly resolved m, 1H, $H_{4'}$), 3.29 (t, 1H, partly overlapped with H_2O), 3.72 (dd, 1H, *J* = 10.8 Hz, 5.6 Hz, $H_{5'}$), 5.15 (br s, 1H, OH), 7.19 (s, 1H, $H_{1'}$), 8.12 (br s, 2H, NH_2), 8.39 (s, 1H, H_8), 11.33 (br s, 1H, NH). ^{13}C NMR (100 MHz) 6.8 ($C_{3'}$), 19.8 ($C_{4'}$), 63.6 ($C_{5'}$), 109.4, 110.9, 115.1, 136.7, 152.8, 157.3 ($C_{1'}$, $C_{2'}$, and isoguanine). ESI-MS 256 (M+Na, 100.0), 234 (M+H, 27.5). Anal. Calcd for $C_{10}H_{11}N_5O_2$: C, 51.50; H, 4.75; N, 30.03. Found: C, 51.59; H, 5.00; N, 30.22.

4.7.2. Method B. Using sodium nitrite. Sodium nitrite (1 M, 0.85 mL, 0.85 mmol) was added to a solution of compound **3a** (96 mg, 0.41 mmol) in 80% AcOH (5 mL) and 1 M HCl (1.3 mL, 1.3 mmol) with stirring in the dark at 0 °C. The reaction mixture was then worked up as described in Method A to give compound **11a** (38 mg, 40%) identical to the product obtained by Method A.

4.8. (*R*,*Z*)-9-[2-(Hydroxymethyl)cyclopropylidene]methylisoguanine (11b)

The procedure for *S*-enantiomer **3a** (Method B without HCl) was followed with *R*-enantiomer **4a** (405 mg, 1.7 mmol), 50% AcOH (30 mL), and $NaNO_2$ (0.24 g, 3.4 mmol). Yield 145 mg (37%) of **11b**, mp >240 °C (decomp.), $[\alpha]_D^{20}$ −60.8 (*c* 0.3, water). The UV and ^{1}H NMR spectra were identical to those of the *S*-enantiomer **11a**. Calcd for $C_{10}H_{11}N_5O_2$: C, 51.50; H, 4.75; N, 30.03. Found: C, 51.18; H, 5.18; N, 29.90.

4.9. Reaction of the *S*-enantiomer 11a with reagent 17

A solution of compound **11a** (70 mg, 0.30 mmol) and *N*-methylimidazole (250 μL, 4.6 mmol) in pyridine (6 mL) was cooled to 0 °C under N_2 in the dark. Phosphorochloridate reagent **17** (0.38 mmol, 1.05 mL, and 100 mg/mL solution in THF)[4] was then added dropwise with stirring, which was continued at room temperature for 10 h. The solvent was removed in vacuo at room temperature and the residue was chromatographed using CH_2Cl_2–MeOH (9:1) to give product **18** (38 mg, 27%). UV max (EtOH) 263 nm (ε 17,900), 224 (ε 36,800), 209 (ε 41,100). ^{1}H NMR δ 1.17 and 1.24 (partly overlapped m and t, 4H, $H_{3'}$ + CH_3), 1.50 (t, 1H, *J* = 8.4 Hz, $H_{3'}$), 2.15 (m, 1H, $H_{4'}$), 3.31 (m, 1H), 3.73 (dd, 1H, *J* = 8.4 Hz, 5.6 Hz, $H_{5'}$), 3.64 (s, 3H, OCH_3), 4.19 (m, 1H, CH of Ala), 5.15 (br s, 1H, OH), 6.19 (m, 1H, NH of Ala), 6.90 (s, 1H, $H_{1'}$), 7.18, 7.25, 7.37 (3m, 5H, Ph), 7.66 (br s, 2H, NH_2), 8.695, 8.701 (2s, 1H, H_8). ^{31}P NMR −1.92, −1.81. ESI-MS 971 (2M+Na, 19.7), 497 (M+Na, 29.0), 475 (M+H, 15.0), 83 (100.0).

4.10. (*S*,*Z*)-{[(2-Hydroxymethyl)cyclopropylidene]methyl}isoguanine (methylphenylphosphoryl)-*P*-*N*-L-alaninate (11c)

TBN (32 mg, 0.32 mmol) was added to a solution of compound[4] **3b** (50 mg, 0.106 mmol) in 80% AcOH (6 mL). The reaction mixture was stirred in the dark at room temperature for 16 h. The solvent was removed in vacuo at room temperature and the residue was purified by preparative TLC (silica gel, 20 × 20 cm, 2 mm thick layer) developed with CH_2Cl_2–MeOH (9:1) to give **11c** (24 mg, 50%). UV max (EtOH) 299 nm (ε 12,900), 230 (ε 36,400), 208 (ε 36,300). ^{1}H NMR δ 1.15, 1.21 (2d, 3H, *J* = 6.6, 7.2 Hz, CH_3), 1.35, 1.58 (2m, 2H, $H_{3'}$), 2.33 (m, 1H, $H_{4'}$), 3.54, 3.57 (2s, 3H, CH_3O), 3.70, 3.83, 4.01, 4.10 (4m, 3H, $H_{5'}$ and CH, Ala), 6.00 (m, 1H, NH, Ala), 7.15 (m), 7.29 (s), 7.31 (m, total 6H, Ph, and $H_{1'}$), 7.88 (br s, 2H, NH_2), 8.11 and 8.12 (2s, 1H, H_8), 10.79 (br s, 1H, NH). ^{13}C NMR 7.7 ($C_{3'}$), 17.3 ($C_{4'}$), 20.3, 20.4 (CH_3), 50.4 (CH, Ala), 52.6 (CH_3O), 68.5 ($C_{5'}$), 109.0, 112.0, 112.9, 120.9, 120.8, 121.0, 125.2, 130.2, 136.3, 151.3, 156.8 ($C_{1'}$, $C_{2'}$, Ph and isoguanine), 174.4 (CO). ^{31}P NMR 4.41, 4.51. ESI-MS 971 (2M+Na, 28.1). Anal. Calcd for $C_{20}H_{13}N_6O_6P{\cdot}0.8H_2O$: C, 49.14; H, 5.07; N, 17.19; P, 6.33. Found: C, 49.42; H, 5.10; N, 17.14; P, 6.72.

4.11. (*R*,*Z*)-{[(2-Hydroxymethyl)cyclopropylidene]methyl}isoguanine (methylphenylphosphoryl)-*P*-*N*-L-alaninate (11d)

Compound[4] **4b** (0.47 g, 1 mmol) was treated with TBN (0.3 g, 3 mmol) in 80% AcOH (40 mL) as described above for pronucleotide **10c** (reaction time 60 h). Column chromatography on silica gel in CH_2Cl_2–MeOH (95:5 to 9:1) gave **11d** as an amorphous product (325 mg, 68%). An analytical sample was obtained by preparative TLC as described for **11c**. ^{1}H NMR δ 1.17, 1.19 (2d, *J* = 6.4, 7.2 Hz, CH_3), 1.34, 1.60 (2m, 2H, $H_{3'}$), 2.30, 2.37 (2m, 1H, $H_{4'}$), 3.54 (s, 3H, CH_3O), 3.71−3.80, 4.09 (2m, 3H, $H_{5'}$, CH, Ala), 6.00 (m, 1H, NH, Ala), 7.12–7.35 (cluster of m, 6H, Ph, $H_{1'}$), 7.91 (br s, 2H, NH_2), 8.12, 8.14 (2s, 1H, H_8), 11.07 (br s, 1H, NH). ^{13}C NMR 7.4, 7.7 ($C_{3'}$), 17.25, 17.34 ($C_{4'}$), 20.26, 20.33 (CH_3), 50.2, 50.5 (CH, Ala), 52.5, 52.6 (CH_3O), 68.3, 68.5 ($C_{5'}$), 109.4, 111.9, 112.0, 113.1, 120.90, 120.95, 125.2, 130.2, 130.3, 136.3, 151.3, 157.0 ($C_{1'}$, $C_{2'}$, Ph, and isoguanine), 174.45, 174.49 (CO). ^{31}P

NMR 4.24, 4.68. ESI-MS 971 (2M+Na, 34.7), 497 (M+Na, 100.0), 475 (M+H, 47.9). Anal. Calcd for $C_{20}H_{13}N_6O_6P{\cdot}0.3H_2O$: C, 50.06; H, 4.96; N, 17.52; P, 6.46. Found: C, 50.47; H, 5.02; N, 17.54; P, 5.97.

4.12. Antiviral assays

The antiviral assays were described in detail in the previous communications.[14,18,26] The HCMV (Towne and AD169 strains) were performed in human foreskin fibroblast (HFF) culture using a plaque reduction or cytopathic effect (CPE) inhibition assay. The EBV was assayed in Daudi cells by viral capsid antigen (VCA) ELISA and in H-1 cells by DNA hybridization. The cytotoxicity assays were performed in HFF and CEM cells. For further details, see Table 2.

4.13. Porcine liver esterase assay

PLE (200 units) was added to a stirred mixture of pronucleotide **3b**, **4b**, **11c** or **d** (0.5–1.5 mg) in 0.05 M Na_2HPO_4 (pH 7.4, 0.5 mL). Aliquots were withdrawn at appropriate time intervals and they were analyzed by TLC on silica gel plates in CH_2Cl_2–MeOH (4:1) for the starting pronucleotide and then in 2-propanol–NH_4OH–H_2O (7:1:2) to detect product **19**. The results are shown in Table 3.

4.14. Antitumor assays. Disk diffusion soft agar colony formation drug discovery assay[22]

Tumor cells were seeded in soft agar in 60 mm dishes. Solubilized drug was placed onto Whatman No. 1 filter paper disks (6.5 mm diameter) and allowed to dry. The dried disk was then placed on top of the soft agar midway between the center and the edge of the dish. The plates were incubated at 37 °C for 5–7 days to allow the tumor cells to grow and the drug to diffuse off the disk creating a zone of inhibition of colony formation. The plates were then examined using an inverted microscope. The zone of inhibition was measured from the edge of the disk to the first colony (1 unit = 32 μm). A zone of <150 units indicates an agent of insufficient cytotoxic activity. A difference of at least 250 units between the zone for the leukemia and solid tumor is indicative of a significant differential effect. The results are shown in Table 4.

Acknowledgments

Our thanks are due to L. M. Hrihorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University (D. M. Coleman, Director), for mass spectra. We also thank Benjamin Wei, Julie Breitenbach, and Kathy Borysko for their technical assistance. The work described herein was supported by U.S. Public Health Service program project PO1-AI46390 (J.C.D., E.R.K., and J.Z.), contract NO1-AI30049 (E.R.K.) from the National Institute of Allergy and Infectious Diseases, and RO1-CA-44358 (Y.-C.C.) from the National Cancer, Institute National Institutes of Health, Bethesda, Maryland.

References and notes

1. Zemlicka, J. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K., Ed.; Elsevier: Amsterdam, 2002; p 327.
2. Zemlicka, J.; Chen, X. In *Frontiers in Nucleosides and Nucleic Acids*; Schinazi, R. F., Liotta, D. C., Eds.; IHL Press: Tucker, Georgia, 2004; p 267.
3. Qiu, Y.-L.; Ptak, R. G.; Breitenbach, J. M.; Lin, J.-S.; Cheng, Y.-C.; Drach, J. C.; Kern, E. R.; Zemlicka, J. *Antiviral Res.* **1999**, *43*, 37.
4. Qiu, Y.-L.; Geiser, F.; Kira, T.; Gullen, E.; Cheng, Y.-C.; Ptak, R. G.; Breitenbach, J. M.; Drach, J. C.; Hartline, C. B.; Kern, E. R.; Zemlicka, J. *Antiviral Chem. Chemother.* **2000**, *11*, 191.
5. Chen, X.; Kern, E. R.; Drach, J. C.; Gullen, E.; Cheng, Y.-C.; Zemlicka, J. *J. Med. Chem.* **2003**, *46*, 1531.
6. Janeba, Z.; Francom, P.; Robins, M. J. *J. Org. Chem.* **2003**, *68*, 989, and Refs. [2–13] cited therein.
7. Warrell, R. P., Jr.; Berman, E. *J. Clin. Oncol.* **1986**, *4*, 74.
8. Chun, H. G.; Leyland-Jones, B. R.; Caryk, S. M. *Cancer Treat. Rep.* **1986**, *70*, 1225.
9. Suhadolnik, R. J. *Nucleoside Antibiotics*; John Wiley and Sons: New York, 1970, pp 267–270.
10. Suhadolnik, R. J. *Nucleosides as Biological Probes*; John Wiley and Sons: New York, 1979, pp 60–62.
11. Lutz, M. J.; Horlacher, J.; Benner, S. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 499.
12. Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2608.
13. Davoll, J. *J. Am. Chem. Soc.* **1951**, *73*, 3174.
14. Qiu, Y.-L.; Ptak, R. G.; Breitenbach, J. M.; Lin, J.-S.; Cheng, Y.-C.; Kern, E. R.; Drach, J. C.; Zemlicka, J. *Antiviral Chem. Chemother.* **1998**, *9*, 341.
15. Qiu, Y.-L.; Zemlicka, J. *Synthesis* **1998**, 1447.
16. Chen, X.; Zemlicka, J. *J. Org. Chem.* **2002**, *67*, 286.
17. Chen, X.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 265.
18. Qiu, Y.-L.; Ksebati, M. B.; Ptak, R. G.; Fan, B. Y.; Breitenbach, J. M.; Lin, J.-S.; Cheng, Y.-C.; Kern, E. R.; Drach, J. C.; Zemlicka, J. *J. Med. Chem.* **1998**, *41*, 10.
19. Seela, F.; Wei, C.; Kazimierczuk, Z. *Hel. Chim. Acta* **1995**, *78*, 1843.
20. Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* **2004**, *4*, 371.
21. Zemlicka, J. *Biochim. Biophys. Acta* **2002**, *1587*, 276.
22. Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, J.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic: Boston/Dordrecht/London, 1992; p 33.
23. Hayashi, T.; Kumazawa, H.; Nishikawa, J. JP 2002088082, *Chem. Abstr.* **2002**, *136*, 85821.
24. Brown, G. B.; Weliky, V. S. *J. Org. Chem.* **1958**, *23*, 125.
25. This procedure was sufficient for a complete deacetylation.
26. Kushner, N. L.; Williams, S. L.; Hartline, C. B.; Harden, E. A.; Bidanset, D. J.; Chen, X.; Zemlicka, J.; Kern, E. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 2105.
27. Lee, J.-S.; Paull, K.; Alverez, M.; Hose, C.; Monks, A.; Fojo, A. T.; Bates, S. E. *Mol. Pharmacol.* **1994**, *46*, 627.

28. Corbett, T. H.; Panchapor, C.; Polin, L.; Lowichik, N.; Pugh, S.; White, K.; Kushner, J.; Meyer, J.; Czarnecki, J.; Chinnukroh, S.; Edelstein, M.; LoRusso, P.; Horwitz, J. P.; Grieshaber, C.; Perni, R.; Wentland, M.; Coughlin, S.; Elenbaas, S.; Philion, R.; Rake, J. *Invest. New Drugs* **1999**, *17*, 17.