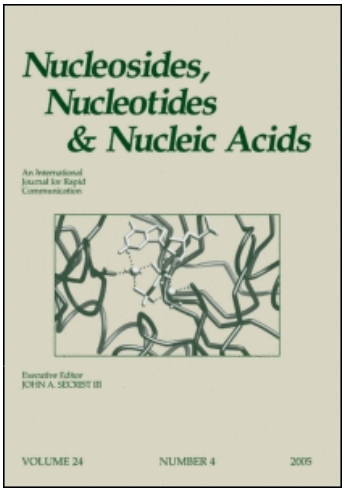

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6-SUBSTITUTED DERIVATIVES OF CARBOVIR: ANTI-HIV ACTIVITY

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Abstract. A series of 6-alkoxy and 6-alkylamino carbovir derivatives were synthesized in order to evaluate prodrug approaches to increased bioavailability of the anti-HIV agent, carbovir. All of the compounds were active against HIV with the N-alkyl derivatives less active than the corresponding O-alkyl derivatives. The adenosine deaminase inhibitor, EHNA, had no effect on the anti-HIV activity of 6-propoxycarbovir, while the adenylic acid deaminase inhibitor, 2′-deoxycoformycin, significantly decreased antiviral activity. These observations suggest that the 6-alkoxycarbovirs are metabolized directly to the monophosphates and are subsequently converted to carbovir monophosphate via adenylic acid deaminase

Introduction

Carbocyclic nucleoside analogs represent an extremely important class of potentially active chemotherapeutic agents. For example, carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine (carbovir) has been identified as a potent and selective inhibitor of HIV-1 replication and cytopathic effects in a variety of human T-lymphoblastoid cell lines.[1,2] A comparison of myelotoxicities of the three antiviral agents, 3'-azido-3'-deoxythymidine (AZT), 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T), and carbovir revealed that carbovir was the least toxic to human and murine hematopoietic progenitor cells, and AZT the most toxic.[3,4] Also, a specific difference between carbovir and other dideoxynucleosides, including AZT, is its relatively low inhibitory activity against DNA polymerase γ.[5,6] It has been suggested that the peripheral neuropathy associated with administration of dideoxynucleosides is due to their inhibition of DNA polymerase γ.[7] For this reason, carbovir and its prodrug forms are undergoing preclinical evaluation for the treatment of AIDS.

We have previously observed that slight changes in the parent molecule can have dramatic effects on biological activity. For example, saturation of the carbocyclic sugar moiety abolished the activity of carbovir.[2] The replacement of the C-8 carbon of the purine with nitrogen also

eliminated activity.[2] However, the substitution of the 6-OH of the purine with NH_2 or H have led to the development of prodrugs with increased bioavailability.[8,9] In order to explore the effects of further modifications of this class of compounds with respect to anti-HIV activity, we have prepared a series of 6-substituted-2-aminopurine analogs.

Chemistry

The racemic 6-substituted carbovirs were obtained directly from the previously described[2] 6-chloro derivative, cis[4-(2-amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl]carbinol, by displacement of the chloro group with the appropriate nucleophile. The (-) enantiomer, **3a**, was prepared from the corresponding (-)-(1S,4R)-4-(2-amino-6-chloro-9H-purin-9-yl)-2-cyclopentenylcarbinol recently described.[9]

Chemical and physical data for new compounds are presented in Table 1.

Results

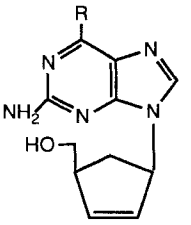
All of the 6-substituted carbovir derivatives exhibited anti-HIV activity in MT-2 cell assays (Table 2). However, the N-alkyl compounds were less active than the corresponding O-alkyl compounds. In addition, the greatest activity appears to reside in the alkyl chains from C_2 to C_4. Substitution with a branched chain (**6**) decreases activity while unsaturation (**7**) retains activity. A comparison of **4** and **8** indicates that the substitution of a methylene group by O severely decreases activity.

Since the anti-HIV activity of racemic carbovir is expressed by the (-) isomer, which has the same absolute stereochemistry as the natural D-ribonucleosides,[10] we prepared the corresponding (-) enantiomer (**3a**) of the O-propyl derivative. As expected, compound **3a** exhibited approximately twice the activity observed for the racemic **3**, confirming that the anti-HIV effect observed for the racemic compounds resides in the (-) enantiomer.

The 6-amino derivative of carbovir (9) also has in vitro activity against HIV. However, the activity of **9** appeared to be solely dependent upon its conversion to carbovir by adenosine deaminase.[10] Thus, it was of interest to examine the possible contribution of adenosine deaminase to the activation of the 6-alkoxy carbovirs. Overnight incubation of **3a** with adenosine deaminase showed no detectable conversion to carbovir or to any other product. Additionally, the antiviral activity of **3a** was not decreased by the addition of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), a potent inhibitor of adenosine deaminase (Table 3). Interestingly, the antiviral activity of **3a** was effectively inhibited by the presence of 2′- deoxycoformycin (dCF). Since dCF is an inhibitor of both adenosine deaminase and adenylic acid deaminase,[11] it appears that adenylic acid deaminase may be involved in the activation of **3a**.

In conclusion, there appears to be a tolerance for a variety of substituents at the 6-position of the anti-HIV agent, carbovir. In contrast to the carbovir prodrug, 6-aminocarbovir, the 6-alkoxy derivatives appear to metabolized directly to the monophosphate and subsequently converted to carbovir monophosphate via adenylic acid deaminase. This route of metabolism is of special interest because the nucleoside form of carbovir is circumvented. It is possible that some of the 6-alkoxy derivative is also metabolized to the 6-alkoxycarbovir triphosphate which subsequently inhibits reverse transcriptase. This possibility is suggested by the observation that the antiviral

Table 1. Chemical and Physical Data for New Compounds.



Cpd·[a]	R	% yield	mp, °C	formula[c]
1	OCH_3	65.2	135-137	$C_{12}H_{15}N_5O_2 \cdot 1/2\ H_2O$
2	OCH_2CH_3	80.0	120-122	$C_{13}H_{17}N_5O_2$
3	$OCH_2CH_2CH_3$	65.5	98-100	$C_{14}H_{19}N_5O_2$
3	$OCH_2CH_2CH_3$	98.0	90-92	$C_{14}H_{19}N_5O_2$
4	$OCH_2CH_2CH_2CH_3$	64.5	112-114	$C_{15}H_{21}N_5O_2$
5	$OCH_2CH_2CH_2CH_2CH_3$	54.7	134-136	$C_{16}H_{23}N_5O_2$
6	$OCH(CH_3)_2$	75.1	190-192	$C_{14}H_{19}N_5O_2$
7	$OCH_2CH{=}CH_2$	44.0	98-102	$C_{14}H_{17}N_5O_2$
8	$OCH_2CH_2OCH_3$	64.5	108-110	$C_{14}H_{19}N_5O_3$
9[b]	NH_2			
10	$NHCH_3$	71.3	218-220	$C_{12}H_{16}N_6O$
11	$NHCH_2CH_3$	66.7	162-164	$C_{13}H_{18}N_6O$
12	$NHCH_2CH_2CH_3$	69.0	157-159	$C_{14}H_{20}N_6O \cdot 1/4H2O$
13	$N(CH_3)_2$	73.4	172-174	$C_{13}H_{18}N_6O$

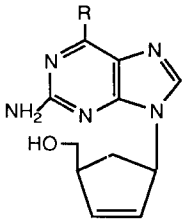
[a]Compound **3a** is the (-)enantiomer, $[\alpha]^{23}_D$ -87.5 (c, 0.30, MeOH). All other compounds are racemic. [b] Ref. 2. [c]C,H,N anal. of all new cpds. were within ± 0.4% theoretical values.

activity of **3a** is not completely inhibited by 10 μM dCF, a concentration sufficient to block all deaminase activity. Metabolism studies with radiolabeled **3a**, and reverse transcriptase inhibition studies with **3a**-triphosphate are underway to answer these questions.

It should be noted that a carbovir derivative, 6-cyclopropylaminocarbovir (1592U89), is undergoing clinical evaluation by Burroughs Wellcome Co.[12] Recent metabolic studies indicate that this compound is activated intracellularly to Carbovir monophosphate by a novel phosphorylation pathway involving the recently characterized enzyme, adenosine phosphotransferase.[13]

Experimental. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained on a General Electric 300. IR spectra were determined with KBr pellets on a Perkin-Elmer 281 spectrometer, and UV spectra were determined on a Beckman DU-

Table 2. Comparative Potency and Selectivity of 6-Substituted Derivatives of Carbovir as Inhibitors of HIV Replication in MT-2 Cells.a-c



Cpd.	R substituent	IC_{50}, μM	TC_{50}, μM
cvr[d]	OH	0.45	>100
1	OCH_3	6.85	>100
2	OCH_2CH_3	5.78	>100
3	$OCH_2CH_2CH_3$	5.88	>100
3a	$OCH_2CH_2CH_3$	2.49	98
4	$OCH_2CH_2CH_2CH_3$	5.21	>100
5	$OCH_2CH_2CH_2CH_2CH_3$	7.03	>100
6	$OCH(CH_3)_2$	9.41	>100
7	$OCH_2CH{=}CH_2$	5.42	>100
8	$OCH_2CH_2OCH_3$	20.0	>100
9	NH_2	12.6	>100
10	$NHCH_3$	>20	>100
11	$NHCH_2CH_3$	11.8	>100
12	$NH_2CH_2CH_2CH_3$	15.5	>100
13	$N(CH_3)_2$	10.6	>100

[a] Experimental details are described in ref. 1. [b]The IC_{50} represents the 50% effective concentration of inhibiting HIV-1 replication. [c]The TC_{50} represents the 50% inhibitory concentration for cell growth. [d] (-) carbovir.

Table 3. Effect of EHNA and 2'-Deoxycoformycin (dCF) on the Anti-HIV Activity of (-) 6-Propoxycarbovir (**3a**) in MT-2 Cells.

Treatment	IC_{50}, μM	TC_{50}, μM
3a	3.08	95.5
3a + 1μM EHNA	2.67	97.6
3a + 10μM EHNA	3.56	95.9
3a + 1μM dCF	7.58	214.2
3a + 10μM dCF	37.7	>300

70-spectrophotometer. Thin-layer chromatography (TLC) was performed on 0.25-mm layers of Merck silica gel 60F-254 and column chromatography was done on Merck 60 (230-400 mesh). Mass spectra were obtained with an AEI Sceintific Apparatus Limited MS-30 mass spectrometer.

Synthesis. Two examples below illustrate general synthetic methods for the preparation of the **(±)-cis-2-amino-9H-9-(4-hydroxymethyl-2-cyclopentene-1-yl)-6-ethoxypurine (2).** A mixture of (±)-cis-[4-(2-amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl]carbinol[2] (250 mg, 0.942 mmol) and sodium hydride (450 mg, 18.8 mmol) in ethanol (25 mL) was heated under reflux for one hour and the cooled mixture was neutralized with acetic acid. The reaction mixture was evaporated in vacuo and the crude product was purified on a silica gel column. Elution with $CHCl_3$ and$CHCl_3$-MeOH (19:1) afforded **2** which was crystallized from EtOAc-ether and gave 207 mg then(80%) of **2**, mp 120-122°C. NMR (DMSO d_6) δ 7.78 (s,1H, 8-H), 6.38 (s, 2H, NH_2, exchangeable), 6.14 (m, 1H, OH, exchangeable), 4.46 (q, 2H, $OC\underline{H}_2CH_3$), 3.46 (t, 2H, 5'-$C\underline{H}_2OH$), 2.89 (m, 1H, 4'-H), 2.65 (m, 1H, CHH), 1.60 (m, 1H, CHH), 1.37 (t, 3H, $OCH_2C\underline{H}_3$). IR (KBr) cm^{-1} 3494, 3311 (NH, OH), 3191 (C=C-H), 2981, 2889 (aliphatic CH), 1631, 1581 (C=C, C=N); MS (EI) (30 EV); 275 (m+), 179 (B+); UV λmax 288, 241, 211 nm (0.1N HCl). Anal. Calc'd for $C_{13}H_{17}N_5O_2$: C,56.73; H, 6.18; N, 25.45. Found: C, 56.86; H, 6.27; N, 25.52.

(±)-cis-[4-(2-Amino-6-methylamino-9H-purinyl-9-yl)-2-cyclopentenyl]carbinol (10). A solution of (±)-cis-[4-(2-amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl]carbinol (100 mg, 0.380 mmol) in aqueous methylamine (5 mL, 40%) was heated at reflux for 1.5h. Evaporation of the solvent in vacuo left a solid residue. The crude product was recrystallized from water and gave 70.5 mg (71.3%) of **10**, mp 218-220°C. NMR (DMSO d_6) δ 7.58 (s, 1H, 8H), 7.26-6.97 (br, 1H, NH, exchangeable), 6.15-5.83 (dd, 2H, CH=CH vinyl), 5.83-5.73 (br, NH_2, exchangeable), 5.46-5.32 (m, 1H, H-1'), 4.82-4.67 (t, 1H, CH_2OH, exchangeable), 3.81-3.37 (m, 1H, CHH'), 1.70-1.48 (m, 1H, CHH'). IR (KBr) cm^{-1} 3486-3210 (NH_2, NH, OH), 2945, 2868 (C-H stretch), 1637,1623, 1595 (C=C, C=N). UV λmax 217, 259, 282 nm in MeOH. Anal. Calc'd for $C_{12}H_{16}N_6O$: C, 55.39; H, 6.20; N, 32.30. Found: C, 55.33; H, 6.18; N, 32.45.

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