Synthesis and Anti-HIV Activity of Novel Cyclopentenyl Nucleoside Analogues of 8-Azapurine

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Novel nucleoside analogues of structure 3—5 were synthesized starting from (±)-*cis*-2-amino-3-cyclopentenylmethanol (1). The chlorine derivative 3 inhibited both HIV-1 and HIV-2 replication in MT-4 cells with IC_{50} values of 10.67 μM and of 13.79 μM, respectively.

Key words carbocyclic nucleoside; 8-azapurine derivative; anti-HIV activity

AIDS is caused by a retrovirus, the human immunodeficiency virus (HIV). Two genetically distinct subtypes (HIV-1 and HIV-2) have been characterized, but it is known that the prime etiological agent of AIDS is HIV-1.[1)]

Nucleoside and their nucleotide analogues have emerged as important therapeutic agents in the treatment of several viral diseases,[2)] including AIDS.[1,2)] These work by blocking the virally-encoded reverse transcriptase (RT).[1)] The majority of nucleoside analogues consist of modifications of the natural substrates in the heterocyclic base or in the sugar moiety.[2)] The most remarkable structural variations are found in the furanose ring, including the elimination of the 2′,3′-hydroxyl groups, the replacement of the oxygen atom by a methylene group (carbocyclic nucleosides),[3,4)] the replacement of tetrahydrofurane by a 1,3-oxathiolane ring,[5)] and the opening of the ring to give acyclic derivatives.[4)] Thus, the last three nucleoside reverse transcriptase inhibitors (NRTIs) approved by FDA for treatment of AIDS are abacavir (a prodrug of carbovir that is a 2′,3′-dideoxy-2′,3′-didehydrocarbonucleoside), emtricitabine (FTC, an oxathiolanyl 2′,3′-dideoxynucleoside analogue), and tenofovir disoproxil (TDF, an acyclic phosphonate nucleoside, and, thus, a nucleotide analogue). So, at this moment eight nucleoside analogues are licensed in United States for clinical use in AIDS.[6)]

Carbocyclic nucleoside analogues have potent and selective anti-HIV activity. In adition, due to the lack of the conventional glycoside linkage, they are stable *in vivo* to cleavage by phosphorylases and hydrolases. Abacavir, the first member of the carbocyclic nucleosides family to be approved for use as a drug, and other 6-modified purine analogues (Fig. 1) have exhibited anti-HIV activity comparable to that of carbovir against HIV-1 (III_B) in MT-4 cells ($IC_{50} \approx 4$ μM).[7)]

Structure–activity relationship studies about carbocyclic nucleoside analogues indicate that a 2-amino-6-substituted purine and a 2′,3′-unsaturated carbocyclic sugar moiety are required for an optimal anti-HIV activity. On the contrary, the substitution of the purine moiety with a pyrimidine, or the replacement of the C8 of the purine with nitrogen, eliminate the activity.[5)] However, the anti-AIDS activity of several acyclic nucleoside phosphonate derivatives of 8-azapurine is known.[8)]

In recent years, we became interested in exploring the therapeutic potential of 1,2-disubstituted carbonucleosides (OTCs), in which the usual 1,3-substitution pattern of the carbocycle is replaced by a 1,2-pattern. Structural theoretical studies using semiempirical methods allow to establish that structural key parameters as the distance between the base and the hydroxyl group or the relative position between the heterocyclic base and the sugar, are very similar to those of natural nucleosides. Initially, we have developed series of purine, and 8-azapurine-based OTCs, with *cis* or *trans* stereochemistry, in which the pseudosugar is a cyclopentane ring.[9,10)] Afterwards, according to the carbovir and 6-modified purine analogues structure, we have introduced an insa turation into the 2′,3′-position of the carbocycle.[11)] Even though some of these compounds have shown significant antiviral activity against certain viruses such as varicella-zoster virus,[9)] they are inactive against HIV, and in most cases, the antitumoral activity was more interesting.[9,10)]

In this paper, considering that 8-azapurine derivatives are a subject of continuous interest in medicinal chemistry,[12,13)] we describe the synthesis and anti-HIV biological activity of a new series of cyclopentenyl nucleoside analogues of 8-azapurine 2,6-disubstituted (compounds **3**—**5**), that present the hydroxymethyl group and the base in *cis* disposition.

Results and Discussion

The carbocyclic nucleoside **3**, precursor of the analogues **4**—**5**, was synthesized by construction of the heterocyclic base from the primary amino group of (±)-*cis*-2-amino-3-cyclopentenylmethanol (**1**),[11)] according to the strategy shown in Chart 1. The triamine intermediate **2** was obtained from **1** in three steps: treatment of **1** with 2-amino-4,6-dichloropyrimidine and triethylamine in *n*-butanol, diazonium coupling at 5 position of the resulting aminopyrimidine by reaction with *p*-chlorobenzenediazonium chloride, and reduction of the diazo linkage (31% overall yield).[11)] The fused triazole ring was then constructed by diazotization of **2** with sodium nitrite in acetic acid, and the spontaneous cyclization of the intermediate diazonium salt to 8-azapurine analogue **3** in 67% yield. Nucleophilic substitution of the 6-chloro atom of **3** by treatment with NaOH gave the 2-amino-6-hydroxy-8-azapurinyl analogue **4** (94% yield). Similarly, reaction of **3** with aqueous ammonia gave the 2,6-diamino-8-azapurinyl analogue **5** (86% yield).

Compounds **3**—**5** were evaluated *in vitro* for their in-

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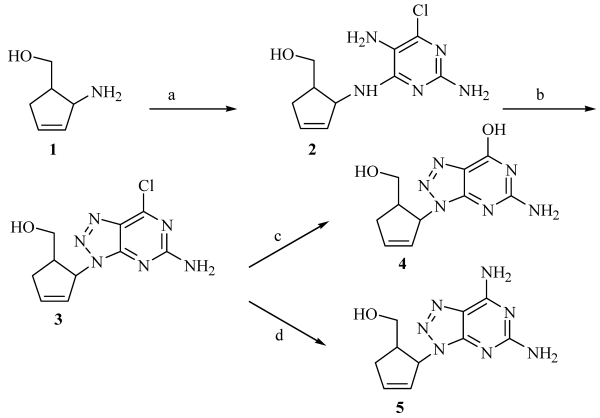
Table 1. Activity of Compounds **3**—**5** against HIV-1 and HIV-2 in MT-4 Cells[a)]

Compd.	IC_{50} (μM), HIV-1 III_B	CC_{50} (μM)	S.I.[b)]	IC_{50} (μM), HIV-2 ROD	CC_{50} (μM)	S.I.[b)]
3	10.67 (±1.2)	51.11 (±0.9)	5	13.79 (±0.9)	51.11 (±0.9)	4
4	>100	>100		>100	>100	
5	>374	374	<1	>477	477	<1

a) Values are average of three experiments (standard deviation is given in parentheses). *b*) *In vitro* selectivity index (CC_{50}/IC_{50}).



Reagents and conditions: (a) 2-amino-4,6-dichloropyrimidine, triethylamine, BuOH, reflux; 4-chlorobenzenediazonium chloride, $NaOAc \cdot 3H_2O$, HOAc, H_2O, r.t.; Zn, HOAc, H_2O–EtOH, reflux (31%); (b) $NaNO_2$, H_2O, AcOH, 0 °C (67%); (c) 0.3 M NaOH, reflux (94%); (d) 25% NH_4OH, reflux (86%).

Chart 1

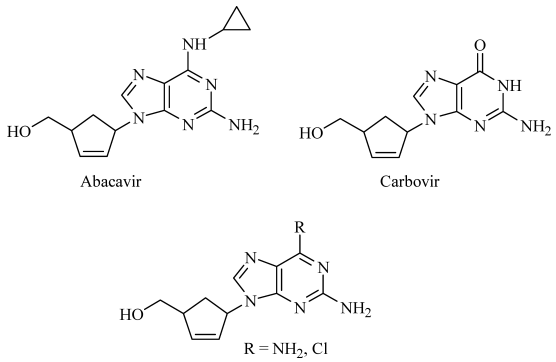


Fig. 1. Carbocyclic Nucleoside Analogues with Anti-AIDS Activity

hibitory effect on the replication of HIV-1 and HIV-2 as well as their cytotoxicity in MT-4 cells using previously established procedures.[14)] The results are summarized in Table 1.

In this study, it was found that the 6-chloro derivative **3** inhibited replication of HIV-1 and HIV-2 at subtoxic concentration. This compound has showed specific activity against the two subtypes of HIV with a quite small selectivity index.

It can be highlighted that the 6-chloro derivative **3** is the first 1,2-disubstituted carbocyclic nucleoside described with anti-HIV activity comparable to that carbovir, abacavir and the other 6-modified purine analogues (Fig. 1) in the same type of assays, whereas the 6-hydroxy derivative **4** and the 6-amino derivative **5**, structural analogues of carbovir and 6-aminocarbovir, respectively, were inactive. On the other hand, all other compounds studied by our group with the same replacements (Cl, OH, NH_2) in the 6 position of the purine or 8-azapurine ring were also inactive.[10,11)]

The activity of **3** shows the validity and the potentiality of this structural modification (1,3- to 1,2-disubstituted analogues) but it demands the need of a more exhaustive SAR study in this class of nucleoside analogues.

Experimental

General Melting points were determined in a Gallenkamp apparatus. IR spectra (KBr discs) were recorded on a Perkin-Elmer 1640 FTIR spectrometer (ν cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker ARX-400 instrument with TMS as internal standard (chemical shifts in δ values, J in Hz). Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer. Flash chromatography (FC) was performed on silica gel (Merck 60, 230—400 mesh).

(±)-*cis*-2-Amino-8-aza-6-chloro-9-[2-(hydroxymethyl)-4-cyclopentenyl]purine (3) A solution of $NaNO_2$ (31 mg, 0.45 mmol) in H_2O (0,7 ml) was added to a solution of compound **2** (79 mg, 0.31 mmol) and AcOH (0.45 ml) in H_2O (0.79 ml at 0 °C, and the mixture was stirred for 1 h. The precipitate **3** (55 mg, 67%) was recovered by filtration, mp 189—191 °C. IR (KBr) cm^{-1}: 3408, 3319, 3224, 2928, 1642, 1606, 1564, 1513, 1389, 1003, 790, 527. ^{1}H-NMR (DMSO-d_6) δ: 7.58 (s, 2H, NH_2), 6.26 (m, 1H, H4′), 5.87 (m, 1H, H5′), 5.73 (m, 1H, H1′), 4.29 (t, 1H, J=4.5 Hz, D_2O exchange, OH), 3.15 (m, 1H, HCHOH), 2.95 (m, 1H, HCHOH), 2.78 (sext, 1H, J=7.7 Hz, H2′), 2.52 (m, 1H, 1H3′), 2.32 (m, 1H, 1H3′). ^{13}C-NMR (DMSO-d_6) δ: 161.6 (C2), 152.4, 151.9, 136.9 (C4′), 131.0 (C5), 127.9 (C5′), 62.4 (C1′), 60.1 (CH_2OH), 43.4 (C2′) 34.9 (C3′). *Anal.* Calcd for $C_{10}H_{11}ClN_6O$: C, 45.04; H, 4.16; N, 31.51. Found: C, 44.87; H, 4.23; N, 31.32.

(±)-*cis*-2-Amino-8-aza-6-hydroxy-9-[2-(hydroxymethyl)-4-cyclopentenyl]purine (4) A mixture of compound **3** (40 mg, 0.15 mmol) and 0.33 M NaOH (2.14 ml) was refluxed for 20 min. The solvent was evaporated under vacuum and the residue was purified by FC using 95 : 5 CH_2Cl_2/MeOH as eluent to give **4** (35 mg, 94%), mp 274—275 °C. IR (KBr) cm^{-1}: 3309, 3162, 2922, 2851, 1698, 1666, 1610, 1563, 1433, 1381, 1292, 1117, 1031, 798, 580. ^{1}H-NMR (DMSO-d_6) δ: 10.93 (s, 1H, OH aromatic), 6.22 (m, 1H, H4′), 5.84 (m, 1H, H5′), 5.55 (m, 1H, H1′), 4.36 (t, 1H, J=4.7 Hz, OH), 3.04 (m, 1H, HCHOH), 2.96 (m, 1H, HCHOH), 2.71 (sext, 1H, J=7.7 Hz, H2′), 2.5 (m, 1H, 1H3′), 2.33 (m, 1H, 1H3′). ^{13}C-NMR (DMSO-d_6) δ: 156.2, 155.7, 151.8 (C4), 136.9 (C4′), 128.7 (C5′), 124.3 (C5), 62 (C1′), 60.7 (CH_2OH), 44.0 (C2′), 35.6 (C3′). *Anal.* Calcd for $C_{10}H_{12}N_6O_2$: C, 48.38; H, 4.87; N, 33.85. Found: C, 48.51; H, 4.65; N, 33.71.

(±)-*cis*-2,6-Diamino-8-aza-9-[2-(hydroxymethyl)-4-cyclopentenyl]-purine (5) A mixture of compound **3** (45 mg, 0.15 mmol) and 25% NH_4OH (6 ml) was refluxed for 1 h. The solvent was evaporated under vacuum and the residue purified by FC using 94 : 6 CH_2Cl_2/MeOH as eluent to give **5** (36 mg, 86%), mp 230—231 °C. IR (KBr) cm^{-1}: 3525, 3424, 3357, 3321, 3164, 2923, 2853, 1678, 1596, 1484, 1421, 1365, 1108. ^{1}H-NMR (DMSO-d_6) δ: 7.52 (s, 2H, D_2O exchange, NH_2), 6.33 (s, 2H, D_2O exchange, NH_2), 6.21 (m, 1H, H4′), 5.86 (m, 1H, H5′), 5.60 (m, 1H, H1′), 4.33 (t, 1H, J=4.9 Hz, OH), 2.97 (m, 2H, CH_2OH), 2.71 (sext, 1H, J=7.6 Hz, H2′), 2.51 (m, 1H, 1H3′), 2.39 (m, 1H, 1H3′) ppm. ^{13}C-NMR (DMSO-d_6) δ: 162.6 (C2), 156.1 (C6), 151.5 (C4), 135.9 (C4′), 128.3 (C5′), 119.9 (C5), 61.4 (C1′), 60.1 (CH_2OH), 43.5 (C2′), 35.2 (C3′). *Anal.* Calcd for $C_{10}H_{13}N_7O$: C, 48.58; H, 5.30; N, 39.65. Found: C, 48.71; H, 5.43; N, 39.75.

Biological Activity Assays The methodology of the anti-HIV assays has been described previously.[14)]

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