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Synthesis and biological evaluation of 1,2-disubstituted carbonucleosides of 2-amino-6-substituted purine and 8-azapurine

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Abstract

One, two-disubstituted carbocyclic nucleoside analogues bearing a 2-amino-6-substituted (chloro, hydroxy or amino) purine or 8-azapurine base were prepared by constructing the base about (±)-2-aminocyclopentane methanol, and their activities against a selection of viruses and tumor cells were determined *in vitro*.

In the search for more potent and selective chemotherapeutic agents, much attention has been focused on nucleoside analogues.[1-3] Among these, carbocyclic nucleosides, in which a methylene or methine group replaces the furan oxygen, often have interesting biological activities.[4] In particular, many such analogues bearing 2-amino-6-substituted purinyl bases have shown antiviral and/or antitumor activity (e.g. carbovir and carbocyclic 2,6-diamino purinylribo- and arabinofuranosides).[5-7]

In this work, in continuation of our work on one, two-disubstituted carbonucleosides (OTCs - cyclopentanes with an hydroxymethyl group and the heterocyclic base attached to contiguous ring carbons),[8] we examined the therapeutic potential of 2-amino-6-substituted purinyl and 8-azapurinyl OTCs **3-8** (Scheme 1). We began with a preliminary theoretical study using the semi-empirical quantum-mechanical method AM1,[9] as implemented by the AMPAC program.[10] The results indicated that the energy-minimized conformations of **3-8** corresponded closely to those of natural nucleosides. Moreover, comparison of the geometry of these conformers with that of the closely related purinyl analogue 2',3'-dideoxyadenosine showed that the root-mean-squared deviation between five key points (the primary hydroxyl group, and N1, N3, N7 and N9 of the purine base)[11] was only 0.15 ± 0.02 Å.

Encouraged by these theoretical results, we proceeded to the synthesis of compounds **3-8** using the routes shown in Scheme 1. In each case, the heterocyclic base was constructed about the primary amino group of the racemic amino alcohol **1**.[12,13] The pyrimidinyl intermediate **2** was obtained from **1** in three steps: treatment of **1** with 2-amino-4,6-dichloro pyrimidine and triethylamine in *n*-butanol; diazonium coupling at position 5 of the resulting aminopyrimidine by reaction with *p*-chlorobenzenediazonium chloride; and reduction of the diazo linkage (66% overall yield). Then, to form the imidazole ring of the purinyl analogues, **2** was treated with triethyl

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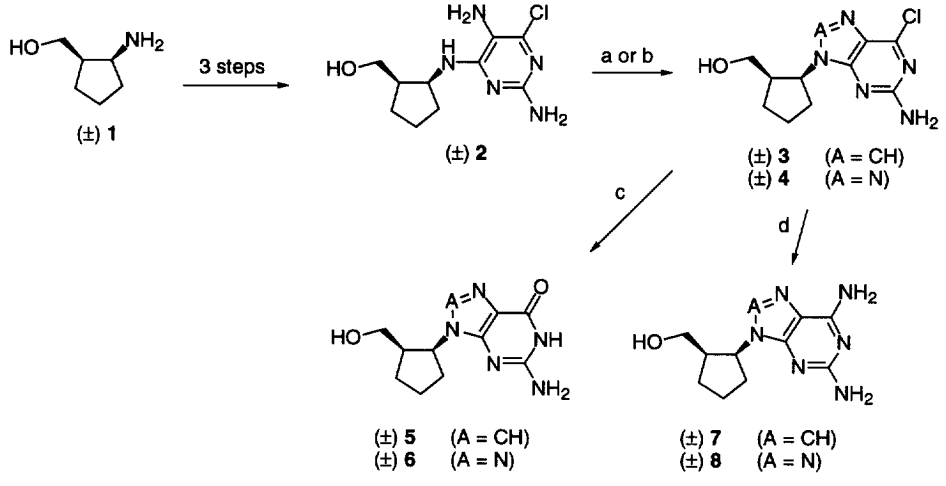
orthoformate in hydrochloric acid, which gave analogue **3** in 76% yield.[14] Similarly, the triazole ring of the 8-azapurinyl analogues was formed by diazotization of **2** with sodium nitrite in acetic acid, the intermediate diazonium salt spontaneously cyclizing to analogue **4** in 51% yield.[15] Nucleophilic substitution of the 6-chloro substituents of **3** and **4** by treatment with sodium hydroxide gave 2-amino-6-hydroxypurinyl analogue **5** (70% yield)[16] and 2-amino-6-hydroxy-8-azapurinyl analogue **6** (72% yield),[17] respectively. Similarly, reaction of **3** and **4** with liquid ammonia gave 2,6-diamino purinyl analogue **7** (89% yield)[18] and 2,6-diamino-8-azapurinyl analogue **8** (90% yield),[19] respectively.

Scheme 1



Reagents. a) $CH(OEt)_3$, 12M HCl, 25°C. b) $NaNO_2$, AcOH, 0°C. c) 0.33M NaOH, reflux. d) NH_3, MeOH, reflux.

The antiviral activities of compounds **3-8** were determined *in vitro* using previously established procedures[20,21] to measure the concentration protecting 50% of the host cells from virus-induced cytopathogenicity (EC_{50}). The viruses and cells used were human immunodeficiency virus (HIV-1 and HIV-2) in human T-lymphocyte (CEM) cells, and varicella-zoster virus (OKA, YS, 07/1 and YS/R strains) and cytomegalovirus in human embryonic lung (HEL) cells. Only the 2,6-diaminosubstituted analogues **7** and **8** showed significant activity against varicella-zoster virus: the purinyl analogue **7** had an EC_{50} of 37 μg/mL against the YS/R strain, and the azapurinyl analogue **8** had EC_{50} of 32, 45 and 50 μg/mL against the OKA, 07/1 and YS/R strains, respectively.

The antitumoral activities of compounds **3-8** against murine leukaemia cells (L1210/0) and human T-lymphocytes (Molt4/C8 and CEM/0) were determined using established procedures for measuring anti-tumor cell activity.[22] *Ara*-A [9-β-(D-arabinofuranosyl)adenine] was included as the reference compound. The IC_{50} values (Table 1) were calculated as the concentration of each compound reducing the number of living cells by 50%. The compounds most active against the tumor cell lines studied were the 2-amino-6-chloroanalogues **3** and **4**, which had IC_{50} values as low as 49 and 53 μM, respectively. The 2,6-diamino purine analogue **7** was up to

three times less potent than analogue **3**, and the guanine analogue **5** was inactive in the concentration range studied. By contrast, the 2,6-diaminoazapurinyl analogue **8** and the azaguanine analogue **6** had similar activity to the 6-chloroazapurinyl analogue **4.**

Table 1.

Antitumor activities of compounds **3-8**

	IC50(μM)[a]		
Compound	L1210	Molt4/C8	CEM
3	71.4 ± 2.5	49.1 ± 7.7	83 ± 18
4	71.0 ± 5.0	75.3 ± 9.9	53.0 ± 12.4
5	>200	>200	>200
6	80.0 ± 3.0	86.0 ± 1.4	86.7 ± 23.0
7	113 ± 10	147 ± 74	90 ± 13
8	77.2 ± 0.6	120 ± 14	90.5 ± 13.4
ara A	14.2 ± 6.4	11.9 ± 7.3	24.8 ± 1.9

[a] 50% Inhibitory concentration, or compound concentration required to reduce proliferation of tumor cells by 50%.

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14. *cis*-2-(2-Amino-6-chloro-9*H*-purin-9-yl)cyclopentylmethanol (**3**). M.p. 162-163° C. IR (KBr disc): 3318, 3204, 2955, 1642, 1608, 1567 cm^{-1}. ^1H NMR (DMSO-d_6): 1.48-2.21 (m, 6H, (-CH_2-)$_3$), 2.34 (m, 1H, -CH-C-O), 3.02 (m, 2H, -CH_2-O), 4.33 (t, 1H, aliphatic -OH, J = 4.80 Hz), 4.82 (q, 1H, -CH-N-, J = 7.25 Hz), 6.85 (bs, 2H, -NH_2), 8.10 (s, 1H, H-8) ppm. Anal. Calcd. for $C_{11}H_{14}ClN_5O$: C, 49.34; H, 5.23; Cl, 13.27; N, 26.17. Found: C, 49.58; H, 5.31; Cl, 13.02; N, 25.98.
15. *cis*-2-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentylmethanol (**4**). M.p. 169-170° C. IR (KBr disc): 3407, 3318, 3222, 1641, 1605, 1563, 1511 cm^{-1}. ^1H NMR (DMSO-d_6): 1.60-2.50 (m, 7H, (-CH_2-)$_3$ + -CH-C-O-), 2.92-3.13 (m, 2H, -CH_2-O), 4.22 (t, 1H, aliphatic -OH, J = 4.85 Hz), 5.14 (m, 1H, -CH-N), 7.58 (bs, 2H, -NH_2) ppm. Anal. Calcd. for $C_{10}H_{13}ClN_6O$: C, 44.69; H, 4.84, Cl, 13.22; N, 31.28. Found: C, 44.62; H, 4.70; Cl, 13.00; N, 31.15.
16. *cis*-2-Amino-6,9-dihydro-9-[2-(hydroxymethyl)cyclopentyl]-1*H*-purin-6-one (**5**). M.p. 297-298° C. IR (KBr disc): 3142, 1713, 1692, 1636, 1607, 1391 cm^{-1}. ^1H NMR (DMSO-d_6): 1.50-2.29 (m, 7H, (-CH_2-)$_3$ + -CH-C-O), 2.97 (m, 2H, -CH_2-O), 4.41 (m, 1H, aliphatic -OH), 4.69 (q, 1H, -CH-N, J = 6.85 Hz), 6.44 (bs, 2H, -NH_2), 7.63 (s, 1H, H-8), 10.58 (bs, 1H, aromatic -OH) ppm. Anal. Calcd. for $C_{11}H_{15}N_5O_2$: C, 53.01; H, 6.02; N, 28.11. Found: C, 53.38; H, 5.99; N, 27.80.
17. *cis*-5-Amino-6,7-dihydro-3-[2-(hydroxymethyl)cyclopentyl]-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (**6**). M.p. > 330° C. IR (KBr disc): 3568, 3333, 3144, 1730, 1705, 1626, 1578 cm^{-1}. ^1H NMR (DMSO-d_6): 1.56-2.40 (m, 7H, (-CH_2-)$_3$ + -CH-C-O), 2.09 (m, 2H, -CH_2-O), 4.28 (m, 1H, aliphatic -OH), 4.98 (q, 1H, -CH-N, J = 6.45 Hz), 6.88 (bs, 2H, -NH_2), 10.91 (bs, 1H, aromatic -OH) ppm. Anal. Calcd. for $C_{10}H_{14}N_6O_2$: C, 48.00; H, 5.60; N, 33.60. Found: C, 47.90; H, 5.64; N, 33.46.
18. *cis*-2-(2,6-Diamino-9*H*-purin-9-yl)cyclopentylmethanol (**7**). M.p. 233-234° C. IR (KBr disc): 3349, 1667, 1624, 1597 cm^{-1}. ^1H NMR (DMSO-d_6): 1.44-2.29 (m, 7H, (-CH_2-)$_3$ + -CH-C-O), 2.83-3.07 (m, 2H, -CH_2-O-), 4.70 (m, 1H, -CH-N), 5.81 (bs, 2H, -NH_2), 6.69 (bs, 2H, -NH_2), 7.63 (s, 1H, H-8) ppm. Anal. Calcd. for $C_{11}H_{16}N_6O$: C, 53.22; H, 6.45; N, 33.87. Found: C, 53.18; H, 6.58; N, 33.57.
19. *cis*-2-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentylmethanol (**8**). M.p. 206-207° C. IR (KBr disc): 3337, 3177, 1673, 1625, 1589 cm^{-1}. ^1H NMR (DMSO-d_6): 1.51-2.43 (m, 7H, (-CH_2-)$_3$ + -CH-C-O), 2.85-3.04 (m, 2H, -CH_2-O), 4.42 (t, 1H, aliphatic -OH, J = 5.40 Hz), 4.99 (q, 1H, -CH-N, J = 6.55 Hz), 6.36 (bs, 2H, -NH_2), 7.49 (bs, 2H, -NH_2) ppm. Anal. Calcd. for $C_{10}H_{15}N_7O$: C, 48.19; H, 6.02; N, 39.35. Found: C, 48.03; H, 6.20; N, 39.22.
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