Carbocyclic Analogues of Nucleosides from bis-(Hydroxymethyl)-cyclopentane: Synthesis, Antiviral and Cytostatic Activities of Adenosine, Inosine and Uridine Analogues

José Manuel BLANCO,*a Olga CAAMAÑO,a Franco FERNÁNDEZ,a José Enrique RODRÍGUEZ-BORGES,b Jan BALZARINI, and Erik DE CLERQc

* Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago de Compostela; Santiago de Compostela E-15782, Spain; a CIQ, Departamento de Química, Facultade de Ciencias do Porto; Rua do Campo Alegre, 687-4169007 Porto, Portugal; and b Rega Institute for Medical Research, Katholieke Universiteit Leuven; B-3000 Leuven, Belgium. Received May 6, 2003; accepted July 2, 2003

Six new carbocyclic nucleosides were prepared by constructing a purine base (in compounds 9—11) or pyrimidine base (in 6—8) on the amino groups of (±)-(1β,2α,4β)-4-amino-1,2-cyclopentanediethanol (4) and (±)-(1β,3α,4β)-4-amino-1,3-cyclopentanediethanol (5), and their activities against a variety of viruses and tumour cell lines were determined.

Key words carbocyclic nucleoside; adenosine analogue; uridine analogue; antiviral activity; cytostatic activity

Nucleoside analogues that inhibit the reverse transcriptase of human immunodeficiency virus (HIV) continue to be the cornerstone of AIDS therapy.1—3 Furthermore, although side effects limit the use of 3’-azido-3’-deoxythymidine (AZT), 2’,3’-dideoxyinosine (ddI) and 2’,3’-dideoxycytidine (ddC),4—9 and resistance to single agents has emerged,5,6 nucleoside analogues are still the compounds attracting most attention in the search for nontoxic agents capable of selectively inhibiting the replication of HIV and other viruses. In particular, carbocyclic analogues of nucleosides (CANs), which have no labile glycoside link between the base and the carbocycle replacing the sugar of true nucleosides, could offer an attractive in vivo stability advantage over the 2’,3’-dideoxynucleosides, as well as being more lipophilic (and hence potentially more readily absorbed) because of the replacement of the endocyclic oxygen by a methylene group.

Among CANs with anti-HIV activity are the 2’,3’-unsaturated compounds 2’,3’-dihydro-ddA,7 2’,3’-dihydro-2’,3’-dideoxyguanosine (carbovir)9 (1) and abacavir (Ziagen® (2)), which has better oral bioavailability and better penetration into the central nervous system than the two and is currently being used in combination with other antiretroviral drugs to treat HIV infection in adults.7,9—11

However, reports of significant antiviral activity by nucleoside analogues with more than one hydroxymethyl group on the “sugar” ring (whether furan12,13 or cyclobutane14,15) soon led to investigation of a number of bis(hydroxymethyl)cyclopentane derivatives.16—18 It was found that although (3S,4S)- and rac-bis(hydroxymethyl)cyclopentyl adenine 3 are inactive against HIV-1.16,17 (3S,4S)-3 is active against type 1 herpes simplex virus (HSV-1), with an IC50 of less than 0.0001 μg/ml.19

In this work we extended the search for bis(hydroxymethyl)cyclopentanes with antiviral and antitumour activities by preparing and screening seven that were chosen with a view to analysing the influence of various structural parameters on their biological activity. All these compounds were prepared by construction of the heterocyclic base on the amino group of either (±)-(1β,2α,4β)-4-amino-1,2-cyclopentanediethanol (4) or (±)-(1β,3α,4β)-4-amino-1,3-cyclopentanediethanol (5). Six—the uridine analogues 6—8, the inosine analogue 9, and the adenosine analogues 10 and 11 (see Charts 1, 2)—have not been reported previously.

Preparation of the uridine analogues was based on the propenoylurea variant of Shaw’s synthesis of pyrimidine-2,4-[(1H,3H)-diones,20 to which end 3-ethoxypropenyl isocyanate was prepared by means of a two-step procedure that for this kind of analogues has performed better21 than alternative methods.22 Since the isocyanate is unstable, the resulting reaction solution was added, without prior isolation of the isocyanate, to the amino alcohols 4 and 5. The resulting propenoylureas, 12 and 13, respectively, were then cyclized under conventional acidic conditions,22 affording compounds 14 and 7 in practically quantitative yield. These compounds were finally converted into their 5-ido derivatives 6 and 8 by treatment with iodine in a mixture of dioxide and nitric acid.23

Compounds 9—11 were prepared using standard chemistry for purine CANs24 (Chart 1): amino alcohol 4 was condensed with 5-amino-4,6-dichloropyrimidine, the resulting diamine 15 was cyclized with ethyl orthoformate to obtain the key intermediate 16 (in 84% yield from 4), and this 9-substituted 6-chloropurine was further transformed into the final products in yields of 86—98%. Hydrolysis with 1 N HCl gave the inosine analogue 9; nucleophilic substitution by ammonia gave the adenosine analogue 3; and treatment with cyclopropylamine or isopropylamine in refluxing ethanol gave the N-alkyladenosine analogues 10 and 11, respectively.

Using previously established procedures,25,26 the in vitro antiviral activities of compounds 6—11 were determined against a variety of DNA and RNA viruses, and their cytotoxicities for the host cell lines, were assayed in parallel with
the new compounds generally showed no activity against any of these viruses. The antimutagenic activities of compounds 6—11 were assayed against murine leukaemia cells (L 1210/0) and human T-lymphocytes (Molt4/C8 and CEM/0) using procedures described elsewhere.26 The concentrations required to reduce cell growth by 50% (IC50) were >200 μg/ml in all cases.

Experimental

Melting points are uncorrected and were determined in a Reichert Kofler Thermopan or in capillary tubes in a Buechi 510 apparatus. Infrared spectra of samples in KBr discs (solids) or of films between NaCl plates (oils) were recorded in a Perkin-Elmer 1640 FTIR spectrophotometer. 1H-NMR spectra (300 MHz) and 13C-NMR spectra (75 MHz) were recorded in a Bruker AMX 300 spectrometer using tetramethylsilane (TMS) as internal reference (chemical shifts in δ values, J in Hz). Mass spectra were recorded on a Kratos MS-59 spectrometer. Microanalyses were performed in a Perkin-Elmer 240B element analyser by the Microanalysis Service of the University of Santiago. Flash chromatography was performed on silica gel (Merck 60, 230—240 mesh) and analytical TLC on pre-coated silica gel plates (Merck 60 F254, 0.25 mm). Reagents and solvents were supplied by Aldrich Chemical Co. Starting materials 4 and 5 were prepared as previously described.27

(±)-1β,2α,4β,4α-[4-(5-Amino-6-choropyrimidin-4-yl)amino]-1,2-cyclopentanediol (15) A solution of (±)-4 (0.12 g, 0.69 mmol), 5-aminoo-6,6-dichloropyrimidine (0.17 g, 1.04 mmol) and triethylamine (0.9 ml) in dry n-butanol (8 ml) was refluxed under argon for 48 h. After cooling, the solvents were removed under reduced pressure and purification of the residue by column chromatography with 1:1 CHCl3/MeOH as eluent afforded (±)-15 as a viscous paste (0.19 g, 99%). IR (film) νmax: 3359, 3300, 2937, 1704, 1651, 1583, 1505, 1470, 1221 cm−1. 1H-NMR (DMSO-d6), as previously reported.16 13C-NMR (DMSO-d6): δ: 30.61 and 36.65 (C3+5), 38.69 and 47.05 (C1+2), 54.13 (C4), 63.04 (CH2O), 65.63 (CH2O), 123.71 (C5a), 137.42 (C4a), 145.82 (C2aa), 152.98 (C6aa). Electron impact (EI)-MS m/z (%): 273 (12, M+1), 152 (2), 271 (C5a), 256 (20), 241 (33), 144 (100), 127 (6). Anal. Caled for C12H16N4O2: C, 50.89; H, 6.33; Cl, 12.88; N, 19.68. Found: C, 50.85; H, 6.33; Cl, 12.88; N, 19.40.

(±)-1β,2α,4β,4α-[4-(6-Chloro-9H-purin-9-y1)]-1,2-cyclopentanediol (16) A mixture of (±)-15 (0.76 g, 2.79 mmol), triethyl orthoformate (16.2 ml, 144.3 mmol) and 12 N HCl (0.84 ml) was stirred at room temperature for 18 h and then condensed to dryness under reduced pressure. The residue was redissolved in 0.5 N HCl (25 ml), this mixture was brought to pH 7 with 1 N NaOH. The solvents were then evaporated and the semisolid residue was chromatographed on silica gel with 10:1 CH2Cl2/MeOH as eluent, affording (±)-16 as a viscous paste (0.67 g, 85%). IR (film) νmax: 3379, 2937, 1592, 1557, 1494, 1394, 1336, 1200 cm−1. 1H-NMR (DMSO-d6), as previously reported.16 13C-NMR (DMSO-d6): δ: 29.99 and 36.08 (C3+5), 38.74 and 45.33 (C1+C2), 58.90 (C4), 62.70 (CH2O), 65.28 (CH2O), 131.52 (C5a), 147.10 (C6a), 149.31 (C6a), 152.13 (C4a), 151.49 (C3a), 273 (12, M+1), 272 (M−1), 251 (16), 222 (2), 181 (18), 155 (100), 119 (13). Anal. Caled for C14H18ClN6O2: C, 50.98; H, 5.35; Cl, 12.54; N, 19.82. Found: C, 50.89; H, 5.39; Cl, 12.67; N, 19.68.

(±)-6,9-Dihydroxy-1β,2α,4β,4α-[4-(6-Chloro-9H-purin-9-y1)]-1,2-cyclopentanediol (17) A solution of (±)-9 (0.15 g, 0.53 mmol) was refluxed for 4 h in 1 N HCl (15 ml). After cooling, the solvent was removed under reduced pressure, the solid residue (0.28 g) was suspended in water (10 ml), and this suspension was brought to pH 7 with 1 N NaOH. The solvents were then evaporated, and the semisolid residue was chromatographed on silica gel with 10:3 CHCl3/MeOH as eluent, which afforded (±)-9 as a white solid (0.12 g, 86%). An analytic sample was obtained by recrystallization from ethanol. mp 208—210 °C. IR (KBr) νmax: 3384, 2868, 1694, 1591, 1546, 1415, 1340, 1217 cm−1. 1H-NMR (DMSO-d6): δ: 1.75—1.76 (2H, m), 1.80—1.92 (1H, m), 2.20—2.29 (2H, m), 2.56—2.63 (2H, m), 3.34—3.49 (4H, m, 2×CH2O), 4.37—4.58 (3H, m, D2O exchang.), 8.07 and 8.22 (2H, s, 2×H4), 12.30 (1H, brs, δD exchang., 1H, m). 13C-NMR (DMSO-d6): δ: 35.46 and 41.85 (C2+5), 44.03 and 51.07 (C3+5), 43.74 and 45.33 (C1+C2), 58.90 (C4), 62.70 (CH2O), 65.28 (CH2O), 131.52 (C5a), 147.10 (C6a), 149.31 (C6a), 152.13 (C4a). El-MS m/z (%): 283 (1, (M+1)+), 282 (7, M−1), 251 (16), 222 (2), 181 (18), 155 (100), 119 (13). Anal. Caled for C14H18ClN6O3: C, 50.98; H, 5.35; Cl, 12.54; N, 19.82. Found: C, 50.89; H, 5.39; Cl, 12.67; N, 19.68.

Those of standard drugs with known antiviral activities. The viruses and cells used were type 1 herpes simplex virus (strain KOS), type 2 herpes simplex virus (strain G), vaccinia virus, vesicular stomatitis virus and thymidine-kinase-deficient (TK−) KOS in E6SM cells; type 3 parainfluenza virus, type 1 reovirus, Sindbis virus, Coxsackie B4 virus and Punta Toro virus in Vero cells; respiratory syncytial virus in HeLa cells; cytomegalovirus (strains AD-169 and DAVIS), varicella zoster virus (strains OKA and YS) and thymidine-kinase-deficient (TK−) varicella zoster virus (strains 07/1 and YS/R) in human embryonic lung (HEL) cells; and HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells (at compound concentrations up to 100 μg/ml). At subtoxic concentrations...
(3) (2)-16 (0.24 g, 0.85 mmol) was refluxed for 15 min in concentrated HNO3 (29 ml). After cooling, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel with 4:1 EtOAc/MeOH as eluent, which afforded (2)-3 as a white solid (0.2 g, 91%). An analytic sample was obtained by recrystallization from ethanol. mp 182—184 °C (lit.,15 mp 182—183 °C). IR (KBr) νmax = 3252, 2930, 2344, 1618, 1527, 1440, 1381, 1236, 1130 cm−1. 1H-NMR (DMSO-d6) δ: 0.56—0.65 (2H, m, cyclopropyl CH2), 0.67—0.73 (2H, m, cyclopropyl CH2), 0.91—1.03 (1H, m), 2.59—2.61 (1H, m), 2.95—3.11 (1H, m), 3.27—3.33 (2H, m, CH3O), 3.39—3.41 (2H, m, CH3O), 4.54—4.63 (3H, m, 4-H(CH2O) dioxane exchang.), 7.80—7.81 (1H, m, NH), 8.19 and 8.21 (2H, 2H (2-H, m, 9.18—9.26 (1H, m), 10.38—10.44 (1H, m), 10.59—10.69 (1H, m), 11.40—11.50 (1H, m), 11.74—11.90 (1H, m). 13C-NMR (DMSO-d6) δ: 31.54 and 31.62 (C4), 36.89 and 37.09 (C3), 49.81 (C2), 60.37 (CH2O), 65.00 (CH2O), 101.60 (CH2O), 141.11 (C5), 144.52 (C6), 150.24 (C7), 156.22 (C8). El-Ms %: 286 (30, M+1), 285 (29, M+1), 199 (25), 190 (24), 173 (23), 147 (22), 131 (21), 115 (20), 103 (19), 92 (18), 71 (17). Anal. Calc. for C12H17N5O2: C, 54.74; H, 6.51; N, 15.11. Found: C, 54.63; H, 6.35; N, 15.10. A mixture of (2)-14 (0.18 g, 9.8%) as a viscous paste. IR (film) νmax = 3382, 3300, 2939, 1684, 1466, 1385, 1268 cm−1. 1H-NMR (DMSO-d6) δ: 30.15 and 34.58 (C2 (5), 38.08 and 44.37 (C3 (4), 58.56 (C1), 63.00 (CH3O), 65.37 (CH3O), 101.62 (CH=CH), 143.12 (CH=CH), 153.22 (NCONH), 163.21 (NCONHOH). El-Ms % (m/z): 241 (6, (M+1)−), 240 (15, (M)−), 192 (4), 151 (5), 113 (100). Anal. Calc. for C13H19N5O2: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.11; H, 6.80; N, 11.64.

(3) (2)-18 (0.24 g, 0.85 mmol) was refluxed for 30 min, allowed to cool, brought to pH 7 with 2N NaOH, and concentrated to dryness. The residue was extracted with ethanol (3×30 ml) and the concentration of the extracts left a residue that upon purification on silica gel with 5:1 EtOAc/MeOH as eluent afforded (3)-(18 (0.18 g, 9.8%) as a white solid.)

(3) (2)-19 (0.24 g, 0.85 mmol) was refluxed for 30 min, allowed to cool, brought to pH 7 with 2N NaOH, and concentrated to dryness. The residue was extracted with ethanol (3×30 ml) and the concentration of the extracts left a residue that upon purification on silica gel with 5:1 EtOAc/MeOH as eluent afforded (3)-(19 (0.18 g, 9.8%) as a viscous paste. IR (film) νmax = 3382, 3300, 2939, 1684, 1466, 1385, 1268 cm−1. 1H-NMR (DMSO-d6) δ: 30.15 and 34.58 (C2 (5), 38.08 and 44.37 (C3 (4), 58.56 (C1), 63.00 (CH3O), 65.37 (CH3O), 101.62 (CH=CH), 143.12 (CH=CH), 153.22 (NCONH), 163.21 (NCONHOH). El-Ms % (m/z): 241 (6, (M+1)−), 240 (15, (M)−), 192 (4), 151 (5), 113 (100). Anal. Calc. for C13H19N5O2: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.11; H, 6.80; N, 11.64.

(3) (2)-20 (0.24 g, 0.85 mmol) was refluxed for 30 min, allowed to cool, brought to pH 7 with 2N NaOH, and concentrated to dryness. The residue was extracted with ethanol (3×30 ml) and the concentration of the extracts left a residue that upon purification on silica gel with 5:1 EtOAc/MeOH as eluent afforded (3)-(20 (0.18 g, 9.8%) as a white solid.)
(±)-1-(1β,2α,4β)-2,4-bis(Hydroxymethyl)cyclopentyl]-5-ido-1,2,3,4-tetrahydropyrimidine-2,4-dione (8) A mixture of (±)-7 (0.14 g, 0.58 mmol), dioxane (9 ml), I2 (0.3 g, 1.18 mmol) and 0.75 N HNO3 (0.8 ml) was refluxed for 3 h. After cooling, the solvents were removed under reduced pressure and the residue was chromatographed on silica gel with 50:1 EtOAc/MeOH as eluent, which afforded (±)-8 (0.17 g, 85%) as a white solid. mp 80—82 °C. IR (KBr) υmax: 3433, 3404, 2927, 1684, 1604, 1448, 1345, 1271, 1044 cm−1. 1H-NMR (DMSO-OCI). EI-MS m/z (%): 367 (4, (M+1)aa), 366 (29, M+1bb), 365 (4), 239 (100), 195 (31). Anal. Calcd for C11H15IN2O4: C, 36.08; H, 4.13; I, 34.66; N, 7.73; Found: C, 36.17; H, 4.01; I, 35.01 (C34H31IN2O4). 13C-NMR (DMSO-OCI). 64.48 (CH 2O), 69.11 (Cl), 146.89 (Cl). 4.64—4.72 (3H, m, 1-H). 11.57 (1H, br s, D 2O exchang., CONHCO). 13C-NMR (DMSO-OCI). 64.48 (CH 2O), 69.11 (Cl), 146.89 (Cl). 4.64—4.72 (3H, m, 1-H+2×OH (D 2O exchang.), 8.16 (1H, s, CH=Cl), 11.57 (1H, brs, D2O exchang., CONHCO). 13C-NMR (DMSO-OCI). EI-MS m/z (%): 367 (4, (M+1)aa), 366 (29, M+1bb), 365 (4), 239 (100), 195 (31). Anal. Calcd for C11H15IN2O4: C, 36.08; H, 4.13; I, 34.66; N, 7.73. Found: C, 36.17; H, 4.01; I, 34.52; N, 7.73.