Design, Synthesis, Conformational Analysis and Biological Activities of Purine-Based 1,2-Di-substituted Carbocyclic Nucleosides

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New 1,2-di-substituted carbocyclic nucleosides with 6-chloropurine, adenine and hypoxanthine bases were synthesized by construction of purine on the primary amino group of (±)-trans-2-aminocyclopentylmethanol. AM1 calculations showed close correspondence between the positions of the heteroatoms in the adenine derivative and dideoxyadenosine. The most active of the new compounds in antiviral assays and antitumoral assays against L1210/0, MOLT4/C8 and CEM/0 cells was the 6-chloropurine derivative.

Key words carbonucleosides; purine derivative; AM1 semiempirical method; antiviral agent; antitumor agent

In the past decade, a large number of nucleoside analogues with antiviral and/or antitumoral properties have been successfully designed and synthesized.1—3) Some 2',3'-dideoxynucleosides are currently the drugs of choice for the treatment of certain viral infections (including human immunodeficiency virus (HIV) infection), these work by blocking viral reproduction, thus inhibiting reverse transcriptase.4) Another successful modification has been the replacement of the endocyclic oxygen atom of the nucleoside sugar ring with a methylene group,5) which reduces phosphorylase- and hydrolase-catalyzed reactivity (thereby increasing the in vivo half life)6) and increases lipophilicity (thus favoring absorption and penetration of the cell membrane). The potent HIV-1 inhibitor carbovir combines both these structural modifications.7)

We have recently investigated the properties of 1,2-disubstituted carbocyclic nucleosides (OTCs), in which the hydroxymethyl group of a carbocyclic sugar analogue is substituted at a position adjacent to the nitrogenated base.8) Molecular modeling of the cis isomers of cyclopentane-based OTCs has shown that in the most stable conformers the glycoside linkage is anti ($\chi=-90^\circ$, where for purine aglycons $\chi=C4-N9-C1'-C2'$) and $\gamma$ (C1'–C2'–C1–O2') can, as in most active nucleosides,9,10) be $-60^\circ$ (gg), $+60^\circ$ (gt) or $180^\circ$ (tg).11)

In this paper we report the synthesis, theoretical conformational analysis and preliminary antiviral and antitumoral activities of a new series of cyclopentane-based OTCs in which the hydroxymethyl group is trans to a purine base (adenine, 6-chloropurine or hypoxanthine).

Results and Discussion

As shown in Chart 1, racemic compounds 3, 4 and 5 were synthesized starting from (±)-trans-2-aminocyclopentylmethanol (1), which was separated from a mixture of cis and trans isomers obtained in two steps from commercially available ethyl 2-oxocyclopentylcarboxylate (overall yield 84%).12) The amine 1 was condensed with 5-amino-4,6-dichloropyrimidine in refluxing n-butanol containing triethylamine, affording compound 2 in 71% yield. Ring closure with triethyl orthoformate in an acidic medium then gave an 80% yield of the 6-chloropurine 3, the trans stereochemistry of which was shown by a proton nuclear Overhauser effect (NOE) experiment.13) Compound 3 was treated with a solution of ammonia in methanol to obtain the adenine derivative...
4 in 98% yield, and with hot sodium hydroxide to obtain the hypoxanthine derivative 5 in 94% yield.

Conformational analysis of compounds 3—5 was performed by means of AM1 calculations in which the parameters varied were those with the most influence on the relative positions of the base and the hydroxy group on C1': the dihedral angles $\chi$ and $\gamma$, and the puckers of the cyclopentane ring. Great conformational freedom was shown by the finding that the conformers detected for any given compound differed in energy by no more than about 4 kcal/mol. However, in keeping with published data for similar compounds, the most stable had anti glycoside linkages ($\chi$=−90° or −115°) and hydroxymethyl side chains with gg, gt or tg conformations ($\gamma$=−60°, +60° or 180°). In the conformer with $\chi$=−90° and $\gamma$=+60°, the cyclopentane ring adopted an N conformation and the distances of the OH group from the nitrogen atoms of the base were almost identical to those found in ddA (2',3'-dideoxyadenosine) and in most other active nucleosides. This similarity is illustrated by the root mean square (RMS) distances between corresponding heteroatoms being just 0.2—0.35 Å when this conformer was superimposed on the active conformer of ddA ([M+H]⁺ 54.5 (100%), 61.6 (6%), 124.0, 137.4, 146.3, 152.3. MS m/z: 224 ([M+2]⁺, 11), 242 (M⁺, 32), 221 (37), 169 (15), 146 ([M+2]⁻–H₂O) 29, 144 (M⁺–H₂O) 100, 117 (13), 67 (12). Anal. Calcd for C₉H₁₀N₃O₄: C, 49.89; H, 2.23; N, 23.86. Found: C, 49.62; H, 2.33; N, 23.05.}

Experimental

Chemical Melting points were determined in a Reichert Kofler therman apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a Perkin-Elmer 1640FT spectrometer (ν in cm⁻¹). 1H- and 13C-NMR spectra were recorded on a Bruker AMX 300 NMR spectrometer, using tetramethylsilane (TMS) as an internal standard (δ in ppm, J in Hz). Mass spectrometry was carried out in a Hewlett Packard 5988A spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyser. Flash chromatography (FC) was performed on silica gel (Merck 60, 230—400 mesh).

(2)-trans-5-Amino-6-chloro-4-[2-(hydroxymethyl)cyclopentyl]hypoxanthine (4) Gaseous ammonia was bubbled for 1 h through a solution of 3 (100 mg, 0.39 mmol) in CH₂OH (20 ml) in a steel reactor at 80 °C. The reactor was closed and heated for 20 h in an oven at 60 °C, the solvent was evaporated under vacuum, and the solid residue was purified by flash chromatography on silica gel (CH₃Cl/CH₂OH as an eluent, which gave pure 4 (125 mg, 80%), mp 108—110°C. IR (KBr) cm⁻¹: 3292, 3065, 2953, 1593, 1565, 1394, 1220, 1132, 651. 1H-NMR (CDCl₃): δ 1.64—2.51 (m, 7H, (−CH₂−), (−CH−C−O), 2.44 (t, 1H, −CH₂–O), 3.33 (m, 2H, −CH₂–O), 4.85 (q, J=7.2), 7.33 (t, 1H, −CH−C−O), 8.11, 8.17 (s, each 1H, H-2, H-8). 13C-NMR (CDCl₃): δ 23.0 (4 δ), 32.4 (6 δ), 47.5 (2 δ), 124.0, 137.4, 146.3, 152.3. MS m/z: 224 ([M+2]⁺, 11), 242 (M⁺, 32), 221 (37), 169 (15), 146 ([M+2]⁻–H₂O) 29, 144 (M⁺–H₂O) 100, 117 (13), 67 (12). Anal. Calcd for C₉H₁₀N₃O₄: C, 49.89; H, 2.23; N, 23.86. Found: C, 49.62; H, 2.33; N, 23.05.

(2)-trans-5-Chloro-6-chloro-4-[2-(hydroxymethyl)cyclopentyl]hypoxanthine (5) A mixture of 2 (150 mg; 0.62 mmol), CH₂(OEt)₂ (3.4 ml; 0.02 mol) and conc. HCl (0.04 ml) was stirred for 12 h at room temperature. The solvent was then evaporated under vacuum and the solid residue was redissolved in tetrahydrofuran (THF) (10 ml) and 0.5 M NaCl (13 ml). After 2 h at stirring at room temperature, the mixture was neutralized with 0.5 M NaOH, the solvent was evaporated under vacuum (forming an azetropic mixture with water–toluene) and the solid residue was purified by flash chromatography on silica gel (CH₃Cl/CH₂OH as an eluent, which gave pure 5 (30 mg, 98%), mp 153—155 °C; IR (KBr) cm⁻¹: 3300, 2950, 2871, 1687, 1610, 1577, 1419, 1303, 651. 1H-NMR (CDCl₃): δ 1.84—2.13 (m, 6H, (−CH₂−), 2.50 (m, 1H, −CH−C−O), 3.36 (m, 2H, −CH₂–O), 4.58 (m, 2H, −CH−N, −OH, aliphatic−OH), 7.14 (br s, 2H, −NH₂), 8.11, 8.17 (each s, each 1H, H-1, H-8). 13C-NMR (CDCl₃): δ 23.0 (4 δ), 27.7 (3 δ), 32.5 (5 δ), 47.2 (1 δ), 57.9 (1 δ), 62.6 (6 δ), 119.6 (5 δ), 140.3 (8 δ), 149.8 (4 δ), 152.4 (2 δ), 156.3 (6 δ). MS m/z: 224 ([M+2]⁺, 11), 242 (M⁺, 32), 221 (37), 169 (13), 120 (8), 67 (9). Anal. Calcd for C₉H₁₀N₃O₄: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.54; H, 6.60; N, 29.68.

(2)-trans-5-[2-(Hydroxymethyl)cyclopentyl]hypoxanthine (6) A mixture of 3 (100 mg, 0.39 mmol) and 0.5 M NaOH (5 ml) was refluxed for 5 h. The solvent was then evaporated under vacuum and the solid residue was purified by FC using 98: 2 CH₂Cl₂/MeOH as an eluent, which gave pure 6 (1.5 g, 71%). IR (KBr) cm⁻¹: 3250, 2923, 1650, 1581, 1484, 1475, 1444, 1402. 1H-NMR (DMSO-d₆): δ 1.32—1.98 (m, 7H, (−CH₂−), 3.42 (2H, −CH₂–O), 4.07 (q, J=6.7), 1H, −CH−C−O), 4.59 (s, J=5.14, 1H, aliphatic−OH), 5.07 (br s, 2H, −NH₂), 6.63 (d, J=6.8), 1H, NH−), 7.69 (s, 1H, H-2), 13C-NMR (DMSO-d₆): δ 22.5 (4 δ), 27.3 (3 δ), 32.3 (5 δ), 45.1 (2 δ), 54.5 (1 δ), 61.6 (6 δ), 124.0, 137.4, 146.3, 152.3. MS m/z: 224 ([M+2]⁺, 11), 242 (M⁺, 32), 221 (37), 169 (15), 146 ([M+2]⁻–H₂O) 29, 144 (M⁺–H₂O) 100, 117 (13), 67 (12). Anal. Calcd for C₉H₁₀N₃O₄: C, 49.89; H, 2.23; N, 23.86. Found: C, 49.56; H, 3.06; N, 23.28.

Optimization of theoretical molecular geometries was carried by the AM1 semiempirical quantum mechanical method using the program AMPAC, which was run on an SGI work station. The geometry was optimized by varying the torsion angles $\chi$=−90° and $\gamma$=−60° in 94% yield.

Fig. 1. Superimposition of the Stable Conformers of Compound 4 (non bold) and ddA (bold)
Biological Activity Assays  Assays of antiviral activity and cytotoxicity were carried out in accordance with established procedures.16)

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References

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