CARBOCYCLIC 7-DEAZAGUANINE OXETANOCIN ANALOGUES

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Abstract- The synthesis of two carbocyclic 7-deazaguanosine oxetanocins structurally modified at the C-3' center and lacking the C-2' hydroxymethylene group is described. Neither of these target compounds showed antiviral activity against a battery of DNA and RNA viruses.

INTRODUCTION

Oxetanocin A (1) is a novel naturally occurring nucleoside endowed with an oxetanose sugar moiety. Another oxetanosyl nucleoside derivative, oxetanocin G (2) has been produced from oxetanocin A. Both oxetanocin A and oxetanocin G show potent antiviral, antitumor, and antibacterial activities, including activity against CMV.

Due to the intriguing structure and the potent antiviral activity of oxetanocins (1) and (2) the carbocyclic analogues (3) and (4) have been prepared by several research groups and found to also possess significant antiviral activity. As part of an effort to improve upon the biological properties of the carbocyclic oxetanocins, we have drawn upon our successes with 7-deazapurine derivatives and wish to report results with carbocyclic 7-deazaguanine oxetanocin (5). Derivative (6) is also described as a representative of the "nor series" of carbocyclic nucleosides, which has been the focus of our work for several years.
CHEMISTRY
The preparation of 5 (Scheme 1) began with the ester (7), which was converted into the sulfonate (9) by, first, debenzylation to 8 followed by reaction with benzenesulfonyl chloride in pyridine. Reduction of 9 with calcium borohydride in ethanol gave the primary alcohol (10), which was protected as its benzoyl derivative (11). Reaction of 11 with 2-amino-4-chloro-7H-pyrimidinol[2,3-d]pyrimidine in the presence of sodium hydride yielded 7-(cis-3-hydroxymethylcyclobutyl)-2-amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (12). Debenzylation of 12 to 13 with subsequent acidic hydrolysis completed the synthesis of 5.

Scheme 1

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EtO₂C
   O R
   7, R=H
   8, R=H
   9, R=SO₂Ph

ROCH₂
   OSO₂Ph
   10, R=H
   11, R=Bz

ROCH₂
   12, R=Bz
   13, R=H
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Reaction conditions: a, H₂, 10% Pd on carbon, EtOH, 30 psi; b, benzenesulfonyl chloride, pyridine, 0°C; c, NaBH₄, CaCl₂, EtOH, 0°C; d, benzoyl chloride, pyridine; e, 2-amino-4-chloro-7H-pyrimidinol[2,3-d]pyrimidine, NaH, DMF, 80°C, 2 days; f, 0.4 N NaOH, dioxane; g, 1N HCl, reflux.

Attention then turned to the synthesis of 6 by reacting cyclobutylamine (14) with 5-allyl-2-amino-4,6-dichloropyrimidine to provide pyrimidinylamine (15) (see Scheme 2 on the following page). Compound (15) was then treated with 4-methylmorpholine N-oxide in the presence of a catalytic amount of osmium tetroxide. Upon reaction of the resulting crude diol (16) with sodium periodate, the desired 17 was obtained in high yield. Debenzylation of 17 with boron tribromide in methylene chloride followed by quenching the reaction with methanol yielded 18. Acidic hydrolysis of 18 gave the desired 6.

STRUCTURE ASSIGNMENTS
The structure assignment of compound (5) was accomplished by performing an NOE analysis, which showed a strong response between H-1' (δ 4.58) and H-3' (δ 2.15), Ha-2', and Ha-4' (δ 2.35), proving the α-face syn stereochemical relationship of these four hydrogens. Also, the protons on the exocyclic methylene displayed a strong NOE to H₈-2' and H₈-4' (δ 2.05) but not to Ha-2' and Ha-4'. As a consequence, the exocyclic methylene must be located trans to H-2'a' and H₈a'-4'. This data prove conclusively that H-1' and H-3' are located on the same face of the cyclobutane ring and that the 3'-CH₂OH functional group is on the opposite face and, consequently syn to the heterocyclic base.

A similar NMR analysis was performed on 6. In that regard, a strong NOE was observed between H-1' (δ 4.45) and H-3' (δ 3.95), Ha-2' and Ha-4' (δ 2.70). Irradiation of H-3' demonstrated enhancement of H-1', Ha-2', H₈a'-4' and 3'-OH, whereas no NOE enhancement was seen with H₈-2' and H₈-4'. This information shows that the H-1' and H-3' are located on the same face of the cyclobutane ring of (6) and, by analogy to (5) that the 3'-OH is syn to the heterocyclic base.
BIOLOGICAL

Analogue (5) and (6) failed to display any antiviral activity when screened against a number of viruses, including the herpes viruses, vaccinia virus, vesicular stomatitis, coxsackie B4, polio-1, parainfluenza-3, reo 1, sindbis, semliki forest, and HIV.

EXPERIMENTAL

The glassware used in the reactions was dried overnight in an oven at 100 °C. The reactions were carried out using freshly distilled solvents under anhydrous conditions in an argon or nitrogen atmosphere. Dimethylformamide (DMF) was treated with potassium hydroxide and distilled over MgSO$_4$; methylene chloride (CH$_2$Cl$_2$) was distilled over phosphorous pentoxide and stored over molecular sieves; triethylamine (Et,N) was distilled from calcium hydride and stored over solid potassium hydroxide; and, pyridine was distilled from calcium hydride. Unless otherwise noted, reagents were used as received from the supplier. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm E. Merck silica gel 60-F$_{254}$ precoated silica gel glass plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. The column chromatography purifications were performed on Aldrich flash chromatography silica gel 60 (particle size 0.035-0.07 mm; 220-440 mesh ASTM) by eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (¹H and ¹³C) homogeneous materials. The ¹H and ¹³C NMR spectra and the other 1D and 2D spectra were recorded on either a JEOL FX90Q or Bruker AMX-360 spectrometer in CDCl$_3$ or DMSO-$d_6$ referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). The NMR procedures used for structural analysis of 5
and (6) have been reported previously by us.\textsuperscript{13} Melting point data was obtained using a Mel-Temp capillary melting point apparatus and are uncorrected. The microanalyses were performed by M-H-W Laboratories, Phoenix, AZ on samples that were homogeneous by TLC analysis.

(\pm\text{-})9-(\text{cis-3-Hydroxymethylcyclobutyl})-7-deazaguanine (5). A mixture of ethyl trans-3-benzyloxycyclobutane-1-carboxylate\textsuperscript{14} (7) (3.5 g, 14.9 mmol) and 10\% palladium on carbon (100 mg) in EtOH (50 mL) was hydrogenated under 30 psi of hydrogen overnight. The palladium catalyst was removed by filtration and the filtrate evaporated under reduced pressure to afford ethyl trans-3-hydroxycyclobutane-1-carboxylate (8) as a colorless oil (2.03 g, 94\%), which was used in the next step without further purification: \textsuperscript{1}H NMR (360 MHz, CDCl\textsubscript{3}) \delta 4.54 (m, 1H), 4.14 (q, 2H, J=7.1 Hz), 3.14 (br s, 1H), 3.00 (m, 1H), 2.55 (m, 2H), 2.20 (m, 2H), 1.26 (t, 3H, J=7.1 Hz); \textsuperscript{13}C NMR (90.5 MHz, CDCl\textsubscript{3}) \delta 176.2, 65.3, 60.6, 35.8, 31.1, 14.1.

To a mixture of 8 (0.51 g, 3.54 mmol) and benzenesulfonyl chloride (0.63 g, 3.56 mmol) at 0 °C was added pyridine (10 mL) in small portions over a period of 30 min. The resulting mixture was allowed to stand in the freezer for 20 h, then poured into cold H\textsubscript{2}O (10 mL) and extracted with ether (3 x 20 mL). The ether layers were then combined, washed with 1N HCl (50 mL), then saturated sodium bicarbonate solution (50 mL), and dried (MgSO\textsubscript{4}). The solvent was then evaporated under reduced pressure and the residue purified by column chromatography eluting with hexane/EtOAc (1/1) to give ethyl trans-3-benzenesulfonyloxycyclobutane-1-carboxylate (9) (0.89 g, 88\%) as a colorless oil: \textsuperscript{1}H NMR (90 MHz, CDCl\textsubscript{3}) \delta 8.00-7.85 (m, 2H), 7.69-7.44 (m, 3H), 5.05 (m, 1H), 4.16 (q, 2H, J=7.1 Hz), 3.10 (m, 1H), 2.49 (m, 4H), 1.23 (t, 3H, J=7.1 Hz); \textsuperscript{13}C NMR (22.5 MHz, CDCl\textsubscript{3}) \delta 174.7, 133.8, 129.2, 127.7, 74.6, 65.0, 32.2, 29.9.

An ice-cooled suspension of NaBH\textsubscript{4} (0.33 g) in EtOH (20 mL) was treated with a solution of powdered CaCl\textsubscript{2} (0.5 g, 4.5 mmol) in EtOH (20 mL) and the resulting mixture stirred for 30 min. To this was added a solution of 9 (0.50 g, 3.52 mmol) in EtOH (20 mL) and the temperature gradually raised to rt. The mixture was allowed to stand overnight, at which point the solvent was evaporated in vacuo and the residue dissolved in CHCl\textsubscript{3} and this washed with H\textsubscript{2}O. The organic layer was dried (Na\textsubscript{2}SO\textsubscript{4}) and the solvent removed under pressure to give trans-1-benzenesulfonyloxy-3-hydroxymethylcyclobutane (10) (0.40 g, 93.3\%) as a colorless oil, which was used in the next step without further purification: \textsuperscript{1}H NMR (90 MHz, CDCl\textsubscript{3}) \delta 7.84 (m, 2H), 7.62 (m, 3H), 4.94 (m, 2H), 3.58 (d, 2H, J=4.7 Hz), 2.24-1.95 (m, 5H); \textsuperscript{13}C NMR (22.5 MHz, CDCl\textsubscript{3}) \delta 137.4, 133.7, 129.2, 127.7, 74.6, 65.0, 32.2, 29.9.

A mixture of 10 (1.0 g, 4.13 mmol) and benzoyl chloride (0.68 g, 4.83 mmol) in anhydrous pyridine (20 mL) was stirred at rt for 12 h and then poured into ice H\textsubscript{2}O (30 mL). The solution was extracted with CHCl\textsubscript{3} (3 x 30 mL). The organic layers were combined, washed with 1 N HCl (50 mL), saturated sodium bicarbonate solution (50 mL) and H\textsubscript{2}O (50 mL), and dried (Na\textsubscript{2}SO\textsubscript{4}). The solvent was then evaporated. The residue was co-evaporated with toluene (3 x 30 mL) and purified by column chromatography eluting with hexane/EtOAc (11/1) to give trans-1-benzenesulfonyloxy-3-benzooyloxymethylcyclobutane (11) as a colorless oil (1.10 g, 77\%): \textsuperscript{1}H NMR (90 MHz, CDCl\textsubscript{3}) \delta 8.03-7.84 (m, 4H), 7.65-7.27 (m, 6H), 5.01 (m, 1H), 4.31 (d, 2H, J=5.9 Hz), 2.59 (m, 1H), 2.46-2.16 (m,
A solution of 2-amino-4-chloro-7H-pyrrolo[2,3-d]pyrimidine \( (0.24 \text{ g}, 0.43 \text{ mmol}) \) in anhydrous DMF (6 mL) was treated with NaH (0.04 g, 80% oil dispersion, 1.67 mmol) and maintained with stirring under an argon atmosphere at 50 °C. After 30 min, a solution of 11 (0.50 g, 1.45 mmol) in anhydrous DMF (6 mL) was added and the reaction stirred vigorously at 80 °C for 2 d. The DMF was removed in vacuo and the residue purified by column chromatography eluting with hexane/EtOAc (5/2) to afford 2-amino-9-(cis-3-benzyloxyethylcyclobutyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (12) (0.27 g, 53%) as a white solid, which was used directly in the next step: mp 130-131 °C; \(^1\)H NMR (360 MHz, CDCl\(_3\)) δ 8.12-8.01 (m, 2H), 7.60-7.26 (m, 7H), 6.15 (m, 1H), 4.35 (m, 1H), 2.71 (m, 2H), 2.46 (m, 2H); \(^13\)C NMR (90.5 MHz, CDCl\(_3\)) δ 166.5, 158.3, 152.5, 152.4, 131.1, 129.5, 129.4, 122.9, 110.9, 99.9, 67.5, 45.1, 33.2, 27.4. A solution of 0.4 N NaOH (5 mL, 2 mmol) was added slowly to a solution of 12 (0.20 g, 0.56 mmol) in dioxane (20 mL) and stirred at rt overnight. Without isolating intermediate (13), the solution was acidified with 1 N HCl followed by concentration to 10 mL, washing with CH\(_2\)Cl\(_2\) (5 mL) and refluxed overnight. The solvent was evaporated under pressure, and the residue purified by column chromatography to afford 5 (0.10 g, 76 % from 12) as a solid: mp 263-265 °C; \(^1\)H NMR (360 MHz, DMSO-d\(_6\)) δ 10.27 (s, 1H), 6.92 (d, 1H, \( J=3.5 \text{ Hz} \)), 6.26 (d, 1H, \( J=3.5 \text{ Hz} \)), 6.15 (br s, 2H), 4.80 (m, 1H), 4.58 (m, 1H), 3.45 (d, 2H), 2.34 (m, 2H) 2.15 (m, 1H), 2.05 (m, 2H); \(^13\)C NMR (90.5 MHz, DMSO-d\(_6\)) δ 158.6, 152.2, 149.8, 116.9, 101.5, 99.8, 64.1, 43.7, 32.7, 29.6. Anal. Calcd for C\(_{11}\)H\(_{12}\)N\(_4\)O\(_2\): C, 56.40; H, 6.02; N, 23.92. Found: C, 56.35; H, 6.11; N, 23.86.

5-Allyl-2-amino-4-chloro-6-[(cis-3-benzyloxyethylcyclobutyl)amino]pyrimidine (15). A mixture of cis-3-benzyloxyethylcyclobutylamine \(^{14}\) (14) (1.0 g, 5.65 mmol), 5-allyl-2-amino-4,6-dichloropyrimidine \(^{15}\) (1.15 g, 5.67 mmol) and Et\(_3\)N (5 mL, 32.9 mmol) in 1-butanol (75 mL) was heated at 110 °C under a nitrogen atmosphere for 2 d. At this time, TLC (CH\(_2\)Cl\(_2\)/MeOH, 5/1) showed the disappearance of the reactants. The reaction solution was then evaporated to dryness under reduced pressure and the residue subjected to flash column chromatography eluting with CH\(_2\)Cl\(_2\)/EtOAc (5/2) to afford 15 (1.90 g, 97.6%) as a white solid: mp 97-98 °C; \(^1\)H NMR (90 MHz, CDCl\(_3\)) δ 7.32 (s, 5H), 5.75 (m, 1H), 5.17 (br s, 1H), 5.03 (br s, 2H), 4.42 (s, 2H), 4.15 (m, 1H), 3.89 (m, 1H), 3.82 (d, 2H, \( J=6.6 \text{ Hz} \)), 3.23 (d, 2H, \( J=5.6 \text{ Hz} \)), 2.80 (m, 2H), 1.84 (m, 2H); \(^13\)C NMR (22.5 MHz, CDCl\(_3\)) δ 161.8, 160.7, 157.8, 138.0, 133.9, 128.4, 127.8, 127.7, 116.4, 101.4, 70.4, 66.8, 39.3, 38.3, 30.8. Anal. Calcd for C\(_{16}\)H\(_{21}\)N\(_4\)O\(_2\): C, 62.70; H, 6.14; N, 16.25. Found: C, 62.94; H, 6.29; N, 16.20.

2-Amino-7-(cis-3-benzyloxyethylcyclobutyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (2-amino-6-chloro-9-(cis-3-benzyloxyethylcyclobutyl)-7-deazapurine) (17). To a solution of 15 (0.50 g, 1.45 mmol) and 4-methylmorpholine N-oxide (0.57 g, 60% wt in H\(_2\)O, 2.90 mmol) in THF (25 mL) at 0 °C was added a solution of OsO\(_4\) (0.04 g, 0.15 mmol) in H\(_2\)O (5 mL). The resulting mixture was stirred at rt for 4 h, then quenched with sodium bisulfite (0.560 g, 2.94 mmol) in H\(_2\)O (4 mL). After removal of the volatiles in vacuo, the residue was dissolved in H\(_2\)O and extracted with CHCl\(_3\). The organic extracts were combined, washed and dried (Na\(_2\)SO\(_4\)) and the solvent removed under reduced pressure to give the crude diol (16) as a white solid. This solid was then dissolved in EtOH/H\(_2\)O (2/1, 10 mL) and the resulting solution cooled to 0
°C, after which two equivalents (0.62 g, 2.9 mmol) of NaIO₄ were added portionwise. The solution was stirred for 2 h, at which point the solvent was evaporated under reduced pressure and the residue dissolved in H₂O and extracted with CH₂Cl₂. The organic layers were combined and treated with several drops of concentrated HCl and stirred at rt for 2 h. The solution was then neutralized with concentrated NH₄OH, washed with H₂O, then brine and dried over MgSO₄. Removal of the volatiles under reduced pressure gave a residue which was purified by column chromatography eluting with hexane/EtOAc (5/2) to give 17 (0.43 g, 91%) as a white solid: mp 114-116°C; ¹H NMR (90 MHz, CDCl₃) δ 7.35 (s, 5H), 7.10 (d, 1H, J=3.8 Hz), 6.41 (d, 1H, J=3.8 Hz), 5.03 (br s, 2H), 4.71 (m, 1H), 4.49 (s, 2H), 3.96 (m, 1H), 2.93-2.74 (m, 2H), 2.54-2.33 (m, 2H); ¹³C NMR (22.5 MHz, CDCl₃) δ 158.4, 137.7, 129.9, 128.5, 122.7, 100.2, 70.7, 66.4, 40.5, 38.8. Anal. Calcd for C₁₁H₁₁N₄OCl: C, 62.10; H, 5.21; N, 17.04. Found: C, 62.26; H, 5.31; N, 17.23.

9-(cis-3-Hydroxycyclobutyl)-7-deazaguanine (6). A solution of 17 (0.96 g, 2.93 mmol) in anhydrous CH₂Cl₂ (30 mL) was treated with a solution of 1 N BBr₃ in CH₂Cl₂ (2.5 mL, 2.5 mmol) under nitrogen at -78°C. The solution was allowed to warm to rt and stirred overnight, at which point the reaction was quenched with CH₂Cl₂/MeOH (1/1, 50 mL). The solvent was removed under reduced pressure and the resulting residue was purified by flash column chromatography eluting with 5% MeOH in CH₂Cl₂ to give 2-amino-9-(cis-3-hydroxycyclobutyl)-6-chloro-7H-pyrrolo[2,3-pyridine (18) as a white solid (0.69 g, 100%), which was used in the next step without further purification: mp 168-170°C; ¹H NMR (360 MHz, DMSO-d₆) δ 7.36 (d, 1H, J=3.5 Hz), 6.55 (br s, 2H), 6.25 (d, 1H, J=3.5 Hz), 5.21 (d, 1H, J=6.8 Hz), 4.53 (m, 1H), 4.00 (m, 1H), 2.75 (m, 2H), 2.67 (m, 2H); ¹³C NMR (90.5 MHz, DMSO-d₆) δ 158.7, 151.9, 143.2, 123.1, 112.0, 100.0, 59.0, 40.8, 39.1. A solution of 18 (0.110 g, 0.46 mmol) in 1 N HCl (10 mL) was heated to reflux for 6 h with stirring. The solvent was removed under reduced pressure and the residue azeotroped with absolute ethanol to give a material, which was dissolved in a small amount of H₂O. The solution was neutralized to pH 7 with 6 N NaOH and cooled. The compound was collected by filtration and washed with cold H₂O to give 6 (0.90 g, 89%) as a white solid: mp 292 °C (decomp); ¹H NMR (360 MHz, DMSO-d₆) δ 10.28 (s, 1H), 6.94 (d, 1H, J=3.6 Hz); 6.28 (d, 1H, J=3.6 Hz); 6.16 (br s, 2H), 5.23 (br s, 1H), 4.45 (m, 1H), 3.95 (m, 1H), 2.70 (m, 2H), 2.17 (m, 2H); ¹³C NMR (90.5 MHz, DMSO-d₆) δ 158.7, 152.3, 150.0, 116.7, 101.7, 99.9, 59.0, 41.3, 39.1. Anal. Calcd for C₁₀H₁₂N₄O₂: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.37; H, 5.68; N, 25.22.

REFERENCES


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