Design and Synthesis of the 3′-Aminocarbonylamino, Aminothiocarbonylamino and *N*-(Hydroxy)guanidinyl Derivatives of Thymidine as Potential Anti-HIV Agents

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The 3′-substituted-3′-deoxythymidines **3a** (urea), **3b** (thiourea) and **3c** [*N*-(hydroxy)guanidinyl)] were designed based on the known structure-activity correlations for the active anti-HIV agent, 3′-azido-3′-deoxythymidine (AZT). Hydrolysis of 3′-cyanamido-3′-deoxythymidine (**2**) with ammonium hydroxide in the presence of hydrogen peroxide afforded the 3′-urea analogue **3a**, whereas reaction with hydrogen sulfide in methanol gave the 3′-thiourea derivative **3b**. The 3′-[*N*-(hydroxy)guanidinyl] compound **3c** was synthesized by reaction of the 3′-cyanamide **2** with hydroxylamine.

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The clinical success of 3′-azido-3′-deoxythymidine (**1**, AZT) and related 2′,3′-dideoxyuridines in the treatment of human immunodeficiency virus (HIV) has provided a strong impetus for the synthesis and evaluation of structurally related thymidine analogues with potential anti-HIV activity. AZT (Zidovudine) has been studied extensively in view of its potent HIV inhibitory activity and clinical efficacy [1]. However, serious side effects such as suppression of bone marrow proliferation has rendered a large percentage of patients intolerant to long term treatment with AZT [2].

In order to synthesize more effective compounds, we have attempted to identify relevant structure-activity correlations using AZT as a model compound. Replacement of the 3′-hydroxyl substituent present in thymidine by an azido substituent enhances binding to the nucleotide binding site of reverse transcriptase, the target viral RNA dependent DNA polymerase, and also precludes further DNA chain elongation after incorporation into the nascent strand. The azido group is a 1,3-dipole that may be represented by two resonance structures that delocalize the negative charge over the N-3′α and N-3′γ nitrogen atoms. The electronic properties of the azido group in AZT may be important determinants of its binding affinity to reverse transcriptase and its effective phosphorylation by host kinases to the active 5′-triphosphate. A comparison of the x-ray crystal structures of AZT [3,4] and thymidine 3′-phosphate [5] indicated that N-3′α and N-3′γ nitrogen atoms occupy positions that are similar to those of the corresponding thymidylate 3′-phosphate ester oxygen atoms when the thymine bases are superimposed [3]. Thus, it has been proposed that the charge distribution on the azido group may mimic the charge distribution on the phosphate group, and that these groups are accommodated at the nucleotide binding site present in reverse transcriptase.

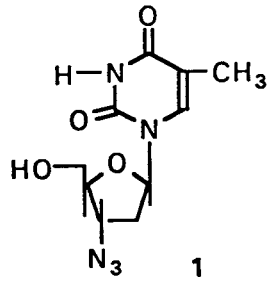

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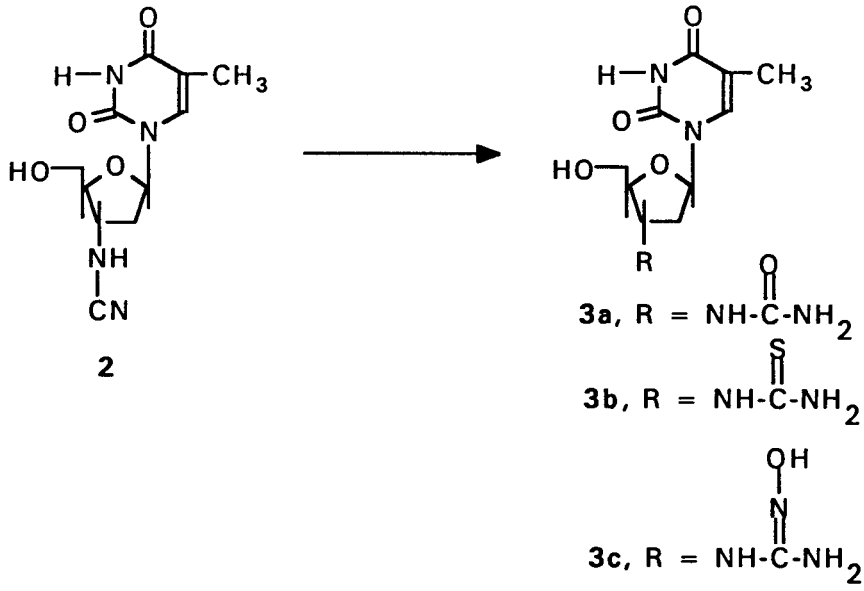
Figure 1

Three new analogues of AZT were therefore synthesized that possess a urea (**3a**), thiourea (**3b**) and *N*-(hydroxy)-guanidinyl (**3c**) substituent at the 3′-position of the sugar ring. These substituents, like the azido substituent in AZT, retain nitrogen atoms at the N-3′α and N-3′γ positions of the 3′-substituent. In addition, a π-electron contributing moiety is present. The resonance contributions for the urea, thiourea and *N*-(hydroxy)guanidinyl substituents may resemble the π-electron delocalization for the phosphodiester oxygen atoms in thymidine 3′-phosphate as well as the azido resonance species [5,6,7]. However, the principle resonance species may maintain a charge distribution that is comparable to that of the azido or phosphate group in AZT or thymidine 3′-phosphate, respectively.

A correlation between the preferred ring sugar conformation and anti-HIV activity for some thymidine analogues has been demonstrated [8]. It has been shown, using the concept of pseudorotation [9] to describe sugar ring conformation, that active analogues adopt pseudorotational phase angles (P) that closely resemble those for the natural substrate thymidine (P = 187.5°) [8]. This observation suggests that the sugar ring conformation may be an important factor for efficient interaction with kinases and/or reverse transcriptase. Therefore, it was of interest to investigate compounds that have a preferred pseudorotational phase angle of greater than 162° (C-2′ endo conformation). The presence of a 3′-*erythro* oxygen, fluoro or nitrogen atom with a negative net atomic charge and positive bond polarities will stablize the sugar ring in

Scheme I



the C-2′ endo or C-3′ exo conformation (P = 164-215°) [8,10]. Therefore, incorporation of a urea, thiourea or *N*-(hydroxy)guanidinyl substituent at the 3′-position would place a non-protonating nitrogen in the α-position of the 3′-substituent which may contribute to stablization of the sugar ring in a conformation that may be favorable for potent anti-HIV activity.

Chemistry.

Reaction of 3′-cyanamido-3′-deoxythymidine (**2**) [11] with cyanamide and ammonium hydroxide in the presence of hydrogen peroxide afforded the 3′-urea derivative **3a** in 76% isolated yield. The thiourea derivative **3b** was prepared in 75% yield by the reaction of **2** with hydrogen sulfide and cyanamide in the presence of triethylamine at 25°. Reaction of **2** with hydroxylamine in methanol at 25° afforded the 3′-[*N*-(hydroxy)guandinyl] product **3c** in 62% yield.

Biological Results.

The *in vitro* anti-HIV activity of 3′-(*N*-(hydroxy)guandinyl]-3′-deoxythymidine (**3c**), determined as the inhibitory concentration required to protect T4 lymphocytes (CEM cell line) against HIV induced cytolysis, was 6.4 x 10^{-4} *M*. This result indicates that **3c** is an inactive anti-HIV agent since this screen is designed to detect agents which interact with virions, cell or virus gene-products to exhibit an antiviral effect that protects cells from cytolysis at any stage of the virus reproductive cycle [12]. Plausible explanations for the inactivity of **3c** could be its failure to undergo phosphorylation by host kinases to the active triphosphate and/or its inability to inhibit reverse transcriptase.

EXPERIMENTAL

Melting points were determined on a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H nmr, ^{13}C nmr) were recorded on a Bruker AM-300 spectrometer using tetramethylsilane as internal standard (^{1}H nmr). The ^{13}C nmr spectra were determined using the J modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Silica gel column chromatography was carried out using Merck 7734 silica gel (25 μ particle size). Thin layer chromatography (tlc) was performed with Whatman MK6F silica gel microslides (25 μ thickness). 3′-Cyanamido-3′-deoxythymidine (**2**) was prepared using the literature procedure [11].

3′-Aminocarbonylamino-3′-deoxythymidine (**3a**).

Ammonium hydroxide (2 ml of 30% w/v) and hydrogen peroxide (2 ml of 30% w/v) were added to a solution of 3′-cyanamido-3′-deoxythymidine (0.2 g, 0.75 mmole) in methanol (3 ml) and the mixture was stirred for 30 minutes at 25°. Tlc indicated that the starting material was consumed. Removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography. Elution with chloroform-methanol (85:15, v/v) afforded **3a** as a white solid (0.161 g, 76%) after recrystallization from methanol, mp 178°; ^{1}H nmr (DMSO-d_6): δ 1.76 (s, 3H, C*H*$_3$), 2.11 (m, 2H, H-2′), 3.49 (m, 2H, H-5′), 3.68 (m, 1H, H-4′), 4.1 (m, 1H, H-3′), 5.07 (t, 1H, O*H*, exchanges with deuterium oxide), 5.53 (s, 2H, N*H*$_2$, exchanges with deuterium oxide), 6.10 (t, 1H, H-1′), 6.42 (d, 1H, 3′-N*H*, exchanges with deuterium oxide), 7.74 (s, 1H, H-6), 11.30 (s, 1H, uracil N*H*, exchanges with deuterium oxide); ^{13}C nmr (deuterium oxide): δ 13.14 (*C*H$_3$), 38.54 (C-2′), 50.61 (C-3′), 62.15 (C-5′), 85.79 (C-1′ or C-4′), 85.93 (C-4′ or C-1′), 112.32 (C-5), 138.87 (C-6), 152.63 (C-2), 162.67 (NH*C*ONH$_2$), 167.28 (C-4).

Anal. Calcd. for $C_{11}H_{16}N_4O_5 \cdot H_2O$: C, 43.71; H, 6.00; N, 18.53. Found: C, 43.59; H, 6.36; N, 18.72.

3′-Aminothiocarbonylamino-3′-deoxythymidine (**3b**).

3'-Cyanamido-3'-deoxythymidine (0.2 g, 0.76 mmole) was added to a solution of triethylamine (1.6 ml) in anhydrous methanol (15 ml), dry hydrogen sulfide gas was bubbled through the solution for one hour and the reaction mixture was stirred for an additional one hour at 25°. Nitrogen gas was bubbled through the mixture and the solvent was removed *in vacuo* to give a yellow residue which was purified by column chromatography using chloroform-methanol (85:15, v/v) as eluant to afford **3b** as a white solid (0.171 g, 75%) after recrystallization from methanol, mp 167°; ^{1}H nmr (DMSO-d_6): δ 1.78 (s, 3H, C*H*$_3$), 2.21 (m, 2H, H-2'), 3.66 (m, 2H, H-5'), 3.87 (m, 1H, H-4'), 4.65 (m, 1H, H-3'), 5.13 (t, 1H, O*H*, exchanges with deuterium oxide), 6.16 (t, 1H, H-1'), 7.04 (br s, 2H, N*H*$_2$, exchanges with deuterium oxide), 7.79 (s, 1H, H-6), 8.13 (s, 1H, 3'-N*H*, exchanges with deuterium oxide), 11.33 (s, 1H, uracil N*H*, exchanges with deuterium oxide); ^{13}C nmr (DMSO-d_6): δ 12.40 (*C*H$_3$), 27.25 (C-2'), 54.68 (C-3'), 61.74 (C-5'), 83.73 (C-1' or C-4'), 85.58 (C-4' or C-1'), 109.54 (C-5), 136.26 (C-6), 150.59 (C-2), 163.99 (C-4), 182.90 (*C*=S).

Anal. Calcd. for $C_{11}H_{16}N_4O_4S \cdot 1/4H_2O$: C, 43.33; H, 5.30. Found: C, 43.26; H, 5.57.

3'-[*N*-(Hydroxy)guanidinyl]-3'-deoxythymidine (**3c**).

Hydroxylamine hydrochloride (0.528 g, 7.6 mmoles) and sodium carbonate (0.4 g, 3.8 mmoles) were stirred for 15 minutes at 25° in water (10 ml). 3'-Cyanamido-3'-deoxythymidine (0.2 g, 0.76 mmole) was then added and the reaction mixture was stirred for two hours. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography. Elution with chloroform-methanol (7:3, v/v) yielded **3c** as a white solid (0.141 g, 62%) after recrystallization from methanol, mp 103°; ^{1}H nmr (DMSO-d_6): δ 1.77 (s, 3H, C*H*$_3$), 2.29 (m, 2H, H-2'), 3.63 (m, 2H, H-5'), 3.90 (m, 1H, H-4'), 4.24 (m, 1H, H-3'), 5.42 (br s, 1H, O*H*, exchanges with deuterium oxide), 6.15 (t, 1H, H-1'), 7.74 (s, 1H, H-6), 7.78 (br s, 2H, N*H*$_2$, exchanges with deuterium oxide), 8.16 (br s, 1H, 3'-N*H*, exchanges with deuterium oxide), 9.88 (s, 1H, =NO*H*, exchanges with deuterium oxide), 11.36 (s, 1H uracil N*H*, exchanges with deuterium oxide); ^{13}C nmr (deuterium oxide): δ 14.04 (*C*H$_3$), 38.99 (C-2'), 53.13 (C-3'), 62.69 (C-5'), 86.07 (C-1' or C-4'), 87.48 (C-4' or C-1'), 113.99 (C-5), 140.31 (C-6), 154.10 (C-2), 160.90 (*C*=NOH), 169.05 (C-4).

Anal. Calcd. for $C_{11}H_{17}N_5O_5 \cdot H_2O$: C, 41.63; H, 6.04; N, 22.07. Found: C, 41.43; H, 6.17; N, 21.82.

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REFERENCES AND NOTES

[1] R. Yarchoan, H. Mitsuya, C. Myers and S. Broder, *N. Engl. J. Med.*, **321**, 726 (1989).

[2] E. Dournon, S. Matheron and N. Rozenbaum, *Lancet*, **2**, 1297 (1988).

[3] A. Camerman, D. Mastropaolo and N. Camerman, *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 8329 (1987).

[4] G. I. Birnbaum, J. Giziewicz, E. J. Gave, T. Lin and W. H. Prusoff, *Can. J. Chem.*, **65**, 2135 (1987).

[5] N. Camerman, J. K. Fawcett and A. Camerman, *J. Mol. Biol.*, **107**, 601 (1976).

[6] A. Caron and J. Donohue, *Acta, Cryst.*, **17**, 544 (1964).

[7] M. R. Truter, *Acta Cryst.*, **22**, 556 (1967).

[8] P. Van Roey, J. M. Salerno, C. K. Chu and P. F. Schinazi, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3929 (1989).

[9] C. Altona and M. J. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).

[10] N. Camerman, D. Mastropaolo and A. Camerman, *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 3534 (1990).

[11] M. Maillard, A. Faraj, F. Frappier, J.-C. Florent, D. S. Grierson and C. Monneret, *Tetrahedron Letters*, **30**, 1955 (1989).

[12] O. Weislow, R. Kiser, D. Fine, J. Bader, R. Shoemaker and M. Boyd, *J. Natl. Cancer Inst.*, **81**, 577 (1989).