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Synthesis and Antitumor Evaluation of Acyclic 1-[ω-(N'-2-chloroethyl-N'-nitrosoureido)alkyl]thymidine Nucleoside Analogues

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In the preparation of acyclic thymidine nucleoside analogues, K_2CO_3 (or NaH) treated thymine in DMSO was alkylated with ω-chloroalkyl nitrite ($Cl-(CH_2)_n-CN$; n=1, 2, 3, 4) to provide an isomeric mixture of 1-(ω-cyanoalkyl)thymine (**2a-d**) and 1,3-bis(ω-cyanoalkyl)thymine in approximately 5 : 1 ratios. Reduction of the cyano function **2a-d** with $NaBH_4/CoCl_2 \cdot 6H_2O$ gave the corresponding 1-(ω-aminoalkyl)thymine (**3a-d**). The newly formed primary amino function in **3a-d** was directly reacted with 2-chloroethylisocyanate to afford the 1-[ω-(N'2-chloroethylureido) alkyl]thymine (**4a-d**) in good yields. Nitrosation of 1-[5-(N'-2-chloroethylureido)pentyl] thymine (**4d**) with glacial acetic acid and dry $NaNO_2$ powder in anhydrous CH_2Cl_2 gave two types of regioisomeric nitrosoureas, 1-[5-(N'-2-chloroethyl-N'-nitrosoureido)pentyl]thymine (**5d**) and 1-[5-(N'-2-chloroethyl-N-nitrosoureido)pentyl]thymine in approximately 5 : 1 ratios. The in vitro cytotoxicity of the synthesized compounds (**2a-d**, **3a-d**, **4a-d** and **5a-d**) against three cell lines (K-562, P-388 and FM-3A) are measured as IC_{50} values. Compounds **3b** and **4c** showed moderate activities against all three cell lines, and all other compounds were found to be not active.

Key worlds : Acyclic thymidine nucleoside analogues, 1-[4-(N'-2-chloroethyl-N'-nitrosoureido) butyl]thymine, Regioisomeric nitrosoureas, *In vitro* cytotoxicity, Human chronic myelogenous leukemia cell, Mouse lymphoid neoplasma cell, Mouse mammary caruisoma cell, IC_{50}

INTRODUCTION

Several pyrimidine and purine base and nucleoside analogs are important weapons in the anticancer and antiviral chemotherapeutic arsenal. Analogs of pyrimidine nucleosides in which the ribose moiety is replaced by acyclic alkyl chains mimicking the cyclic carbohydrate moiety, have long been considered potentially capable of interfering with the activity of various enzymes for which the natural nucleosides or nucleotides serve as substrates. Among the numerous candidates, the nucleoside analogue, 3'-azido-3'-deoxythymidine (AZT, Zidovudine) is at present the only drug receiving wide clinical usage (Mitsuya, *et al.*, 1985). Despite its efficacy, AZT suffers from serious disadvantages. Side effects include headaches, lowered white cell counts and suppression of bone marrow cell formation (Yarchoan, *et al.*, 1988). These considerations have underlined the urgent need for more potent and less toxic agents as well as to meet the continous challenge of drug resistance.

Therefore it was reasoned that the acyclic nucleosides in which the classical cyclic ribose part is replaced with an acyclic side-chain, might possess interesting biological activities (Kim, *et al.*, 1994a,b). As part of our efforts to discover more useful antitumor agents, we prepared a homologous series of 1-[ω-N'-2-chloroethyl-N'-nitrosoureido)alkyl]thymidine nucleoside anologues (**5a-d**), lacking the normal glycosidic part, but containing a biologically cytotoxic 2-chloroethylnitrosoureido function (Kim, *et al.*, 1994c) instead. These homologues were tested for their in vitro cytotoxicities against three cell lines (K-562, P-388, FM-3A), human chronic myelogenous neoplasma cell (K-562), mouse lymphoid neoplasma cell (P-388) and mouse mammany carunoma cell (FM-3A).

MATERIALS AND METHODS

Melting point were determined on an electrothermal capillary melting point apparatus and uncorrected. TLC was performed on glass plates coated with silicone oxide (silica gel $60F_{254}$) and compounds were

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visualized using a UV lamp. Proton nuclear magnetic and ^{13}C-NMR spectra were obtained with a Varian EM-360 spectrophotometer, Varian Gemini 200 MHz, Brucker AM 300 and DPS 200 (solution in dimethylsulfoxide-d_6 with tetramethylsilane as internal standard). The organic solvents and chemicals were obtained from commercial products and purified by the appropriate methods before use.

General Procedure for the Preparation of 1-(ω-Cyanoalkyl)thymine (2a-d)

A solution of thymine (**1**, 1.5 g, 4.00 mmol) in DMSO (30 ml) was dissolved at 90°C temperature and treated with K_2CO_3 (0.55 g, 4.00 mmol). The reaction mixture was stirred by adding ω-cyanoalkyl chloride (0.253 ml, 4.00 mmol) in small portions during 3~5 hours period. The reaction mixture was evaporated to give oily residues, which were crystallized from an appropriate solvent.

1-Cyanomethylthymine (2a): crystallized from EtOH, mp 205°C, 55% yield. ^{1}H-NMR ($CDCl_3$) δ 1.8 (s, 3H, -CH_3), δ 4.8 (s, 2H, -CH_2-CN), δ 7.6 (s, 1H, =C-H), δ 11.6 (s, 1H, -N-H). IR (KBr) ν (C=O) 1676 cm^{-1}, ν (CN) 2240 cm^{-1}.

1-(2-Cyanoethyl)thymine (2b): crystallized from acetone, mp 190~195°C, 36% yield. ^{1}H-NMR ($CDCl_3$) ν 1.9 (s, 3H, -CH_3), δ 2.8 (s, 2H, N-CH_2), δ 3.95 (t, 2H, -CH_2-CN), δ 7.05 (s, 1H, =C-H), δ 8.3 (s, 1H, N-H). IR (KBr) ν (C=O) 1680 cm^{-1}, ν (CN) 2243 cm^{-1}.

1-(3-Cyanopropyl)thymine (2c): crystallized from acetone, mp 180~185°C, 35% yield. ^{1}H-NMR ($CDCl_3$) δ 1.95 (s, 3H, -CH_3), δ 2.1 (s, 2H, C-CH_2-C), δ 2.45 (t, 2H, N-CH_2-C), δ 3.85 (t, 2H, -CH_2-CN), δ 7.0 (s, 1H, =C-H), δ 8.4 (s, 1H, -N-H). IR (KBr) ν (C=O) 1675 cm^{-1}, ν (CN) 2245 cm^{-1}.

1-(4-Cyanobutyl)thymine (2d): crystallized from acetone, mp 155~158°C, 38% yield. ^{1}H-NMR ($CDCl_3$) δ 1.7 (m, 4H, C-CH_2-CH2-C), δ 2.1 (s, 3H, N-CH_3), δ 2.4 (t, 2H, -N-CH_2-C), δ 3.7 (t, 2H, -CH_2-CN), δ 6.95 (s, 1H, =C-H), δ 9.4 (s, 1H, N-H). IR (KBr) ν (C=O) 1685 cm^{-1}, ν (CN) 2239 cm^{-1}.

General Procedure for the Preparation of 1-(ω-Aminoalkyl)thymine (3a-d)

To a stirred solution of 1-(ω-cyanoalkyl)thymine (**2a-d**) (0.5 g, 3.142 mmol) and $CoCl_2 \cdot 6H_2O$ (6.284 mmol) in CH_3OH (50 ml) was added $NaBH_4$ (1.243 g, 31.42 mmol) in several portions under N_2, and the reaction mixture was stirred for 24 hours at the ice-bath temperature. At the end of the reaction, ammonia solution (28%, 50 ml) was added and the solid was filtered. The filtrate was evaporated and the residue was extracted with $CHCl_3$ (5×300 ml) and dried over $MgSO_4$. Filteration and evaporation gave oily residues which were applied to a column packed with silica gel and the column was eluted with hexane:ethyl acetate (20:1=v/v). The presence of the amino group was detected by the appearance of the violet color by the ninhydrin test and confirmed by the disappearance of the cyano peak (2250 cm^{-1}) in the IR spectra.

1-(2-Aminoethyl)thymine (3a): oil, 15% yield. ^{1}H-NMR ($CDCl_3$) δ 1.6 (s, 2H, -C-NH_2), δ 2.0 (s, 3H, CH_3), δ 3.4 (t, 2H, N-CH_2-C), δ 3.7 (t, 2H, -C-CH_2-N), δ 7.0 (s, 1H, =C-H) δ 8.5 (s, 1H, N-H), IR (KBr) ν (C=O) 1690 cm^{-1}.

1-(3-Aminopropyl)thymine (3b): oil, 13% yield. ^{1}H-NMR ($CDCl_3$) δ 1.1 (m, 2H, C-CH_2-C), δ 1.8 (s, 2H, C-NH_2), δ 2.1 (s, 3H, -CH_3), δ 3.1 (t, 2H, -N-CH_2), δ 3.7 (t, 2H, C-CH_2-N), δ 8.2 (s, 1H, =C-H), δ 9.0 (s, 1H, N-H). IR (KBr) ν (C=O) 1680 cm^{-1}.

1-(4-Aminobutyl)thymine (3c): oil, 1% yield. ^{1}H-NMR ($CDCl_3$) δ 1.5 (s, 2H, -NH_2), δ 1.6 (m, 4H, C-CH_2-CH_2-C), δ 1.9 (s, 3H, -CH_3), δ 3.7 (t, 2H, -N-CH_2), δ 4.0 (t, 2H, -CH_2-NH), δ 7.0 (s, 1H, =C-H), δ 8.0 (s, 1H, N-H). IR (KBr) ν (C=O) 1689 cm^{-1}.

1-(5-Aminopentyl)thymine (3d): oil, 13% yield. ^{1}H-NMR ($CDCl_3$) δ 1.5 (m, 6H, C-$(CH_2)_3$-C), δ 1.7 (s, 2H, C-NH_2), δ 2.0 (s, 3H, -CH_3), δ 3.5 (t, 2H, -N-CH_2-C), δ 3.7 (t, 2H, C-CH_2-N), δ 7.1 (s, 1H, =C-H), δ 9.0 (s, 1H, N-H). IR (KBr) ν (C=O) 1685 cm^{-1}.

General Procedure for the Preparation of 1-[2-(N'-2-Chloroethylureido)ethyl]thymine (4a-d).

To a solution of 1-(ω-aminoalkyl)thymine (**3a-d**) (0.5 g, 2.95 mmol) in anhydrous THF (20 ml) was added slowly 2-chloroethylisocyanate (0.225 ml, 2.95 mmol) in anhydrous THF (20 ml) by a syringe in small portions under N_2, and the reaction mixture was stirred for 1 hour at the ice-bath temperature. The solvent was evaporated to give an oily residue, which was purified by the column chromatography ($CHCl_3$:CH_3 OH (8:1)) to afford a pure oily liquid.

1-[2-(N'-2-Chloroethylureido)ethyl]thymine (4a): oily liquid, 25% yield. ^{1}H-NMR ($CDCl_3$) δ 2.0 (s, 3H, -CH_3), δ 3.2 (t, 2H, -N-CH_2-C), δ 3.5 (m, 2H, CH_2-NH), δ 3.6 (m, 2H, -NH-CH_2-C), δ 3.9 (t, 2H, CH_2-Cl), δ 5.3 (s, 1H, NH-CO-N), δ 5.4 (s, 1H, N-CO-NH), δ 7.0 (s, 1H, =C-H), δ 9.0 (s, 1H, N-H). IR (KBr) ν (C=O) 1635 cm^{-1}.

1-[3-(N'-2-Chloroethylureido)propyl]thymine (4b): oily liquid, 20% yield. ^{1}H-NMR ($CDCl_3$) δ 1.5 (m, 2H, C-CH_2-C), δ 2.0 (s, 3H, -CH_3), δ 3.1 (t, 2H, -N-CH_2-C), δ 3.7 (m, 2H, CH_2-NH), δ 3.9 (m, 2H, NH-CH_2-C), δ 4.0 (t, 2H, CH_2-Cl), δ 5.1 (s, 1H, NH-CO-N), δ 5.2 (s, 1H, -CO-NH), δ 7.1 (s, 1H, =C-H), δ 8.5 (s, 1H, N-H). IR (KBr) ν (C=O) 1636 cm^{-1}.

1-[4-(N'-2-Chloroethylureido)butyl]thymine (4c): oily liquid, 30% yield. ^{1}H-NMR ($CDCl_3$) δ 1.5 (m, 4H, C-CH_2-CH_2-C), δ 1.9 (s, 3H, -CH_3), δ 3.5 (t, 2H, -N-CH_2-C), δ 3.7 (m, 2H, CH_2-NH), δ 3.9 (m, 2H, NH-CH_2-C), δ 4.0 (t, 2H, CH_2-Cl), δ 5.1 (s, 1H, NH-CO-N), δ 5.2

(s, 1H, N-CO-NH), δ 7.0 (s, 1H, =C-H), δ 9.7 (s, 1H, N-H). IR (KBr) ν (C=O) 1632 cm^{-1}.

1-[1-(N'-2-Chloroethylureido)pentyl]thymine (4d): oily liquid, 34% yield. ^{1}H-NMR ($CDCl_3$) δ 1.4 (m, 6H, C-$(CH_2)_3$-C), δ 1.9 (s, 3H, $-CH_3$), δ 3.5 (t, 2H, N-CH_2-C), δ 3.7 (m, 2H, CH_2-NH-CO), δ 3.9 (m, 2H, CO-NH-CH_2), δ 4.0 (t, 2H, CH_2-Cl), δ 5.2 (s, 1H, NH-CO-N), δ 5.3 (s, 1H, N-CO-NH), δ 7.0 (s, 1H, =C-H), δ 8.5 (s, 1H, N-H). IR (KBr) ν (C=O) 1635 cm^{-1}.

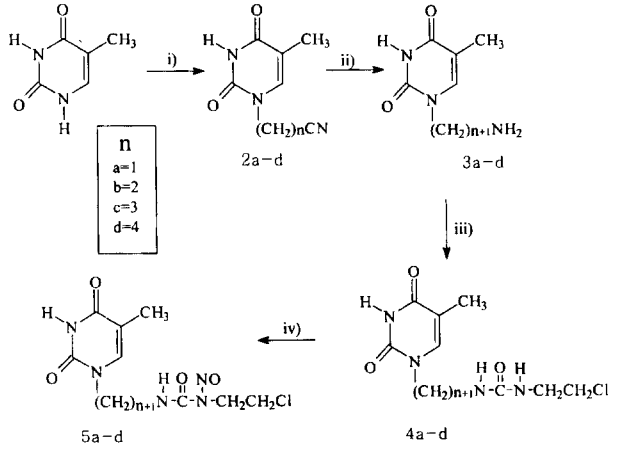
General Procedure for the Preparation of 1-[ω-(N'-2-Chloroethyl-N'-nitrosoureido)alkyl]thymine (5a-d).

A mixture of 1-[ω-(N'-2-chloroethylureido)alkyl]thymine (4a-d) (0.5 g, 2.27 mmol) and dry sodium nitrite (1.6 g, 0.227 mmol) in anhydrous CH_2Cl_2 (50 ml) and glacial acetic acid (20 ml) was stirred for 1 hour at 0°C~5°C, and filtered. The filtrates were evaporated to an oily residue which was chromatographed an silica-gel ($CHCl_3$:CH_3OH=5:1) to give a pure liquid (69% yield); 1-[5-(N'-2-chloroethyl-N'-nitrosoureido)pentyl] thymine and 1-[5-(N'-2-chloroethyl-N-nitrosoureido)pentyl] thymine in an approximately 5:1 ratio; ^{1}H-NMR ($CDCl_3$) δ 1.5 (m, 4H, C-CH_2-CH_2-C), δ 1.9 (s, 3H, $-CH_3$), δ 3.5 (t, 2H, -N-CH_2-C), δ 3.7 (m, 2H, CH_2-NH), δ 3.9 (m, 2H, NH-CH_2-C), δ 4.0 (t, 2H, CH_2-Cl), δ 5.1 (s, 1H, NH-CO-N), δ 7.0 (s, 1H, =C-H), δ 9.7 (s, 1H, N-H). IR (KBr) ν (C=O) 1685 cm^{-1}.

RESULTS AND DISCUSSION

In the preparation of acyclic thymidine nucleoside analogues (Scheme), K_2CO_3-treated thymine in DMSO was alkylated with ω-chloroalkyl nitrile (Cl-$(CH_2)_n$-CN; n=1, 2, 3, 4) to provide an isomeric mixture of 1-(ω-cyanoalkyl)thymine (**2a-d**) and 1,3-bis(ω-cyanoalkyl)thymine in an approximately 5:1 ratio. These results were in accordance with the previous literature report (Montgomery, *et al.*, 1961) describing deoxyribosylation of similar aglycons. Reduction of the nitrile function in compounds **2a-d** with $NaBH_4/CoCl_2 \cdot 6H_2O$, gave the corresponding 1-(ω-aminoalkyl)thymine (**3a-d**). The newly formed primary amino function in **3a-d** was directly reacted with 2-chloroethylisocyanate to afford the 1-[ω-(N'-2-chloroethylureido)alkyl]thymine (**4a-d**) in good yields. Nitrosation of 1-[5-(N'-2-chloroethylureido)pentyl]thymine (**4d**) with glacial acetic acid and dry sodium nitrite powder in anhydrous CH_2Cl_2 gave two types of regioisomeric nitrosoureas, 1-[5-(N'-2-chloroethyl-N'-nitrosoureido)pentyl]thymine (**5d**) and 1-[5-(N'-2-chloroethyl-nitrosoureido)pentyl]thymine in approximately 5:1 ratios. The nitrosating medium of anhydrous formic acid (99% HCOOH) and steric factors can exert some degree of control over the regioisomeric position of the nitrosation reaction (Montgomery *et al.*, 1975). The structural assignments of the nitrosourea compounds (**5a-d**) were based on their IR and ^{1}H-NMR spectra. The IR spectra of these compounds show a band at 1460-1510 cm^{-1}, indicating the presence of a nitroso group.

Furthermore, the sharp absorption at 1710-1745 cm^{-1} is characteristic of the carbonyl absorption by nitrosation of the ureido function (Kim, *et al.*, 1994b,c). The specral asymmetry of the -N(NO)CO-NH-CH_2CH_2Cl (A_2B_2X) system group due to the NH coupling of the adjacent methylene group can be clearly distinguished from the spectral symmetry of the -NHCON(NO)CH_2 CH_2Cl (A_2B_2 system) group. The presence of two dis-



[a]Reagent : i) $Cl(CH_2)_nCN$, DMSO, K_2CO_3(NaH), 60-80℃, 24hrs, ii) $NaBH_4$, $CoCl_2 \cdot 6H_2O$, CH_3OH, 0℃, 24hrs, iii) $ClCH_2CH_2NCO$, CH_2Cl_2, N_2, 0℃, iv) $NaNO_2$/AcOH, 0℃ CH_2Cl_2, 2hrs.

Scheme

Table I. IC_{50} Values of Acyclic Thymidine Nucleoside Analogues (**2a-d, 3a-d, 4a-d, 5a-d**)

Comp. NO	IC_{50} (μg/ml)[a]		
	K-562[b]	P-388[c]	FM-3A[d]
2[a]	>100	>100	>100
2[b]	>100	>100	>100
2[c]	>100	>100	>100
2[d]	>100	>100	>100
3[a]	2.3	26	14
3[b]	5.3	9.3	10
3[c]	45	34	21
3[d]	>100	>100	>100
4[a]	>100	>100	>100
4[b]	50	43	32
4[c]	8.0	4.3	4.7
4[d]	52	42	28
5[a]	8.8	20	19
5[b]	24	38	39

[a]mean values of triplicate runs. The concentration of synthesized compounds required to reduce cell numbers to 50% of controls in a growth inhibition assay.
[b]Human chronic myelogenous neoplasma cell.
[c]Mouse leukemia cell.
[d]Mouse mammary carcinoma cell.

tinct triplets (A_2B_2 system) centered at 3.36 and 4.74 in the ^{1}H-NMR spectra was strong evidence that the nitroso group was attacked to the same hydrogen as the chloroethyl group (-N(NO)CH_2CH_2Cl).

All the acyclic thymine nucleoside analogues were evaluated for antitumor efficacy against the following cell lines; a) human chronic myelogenous leukemia cell (K-562); b) mouse lymphoid neoplasma cell (P-388) and c) mouse mammary carcinoma cell (FM-3A). The cytotoxicity of the synthesized compounds (**2a-d**, **3a-d**, **4a-d** and **5a-d**) against three cell lines measured as IC_{50} values are given in table. Compound **3b** and **4c** showed moderate activities against all three cell lines, and all other compounds were found to be inactive.

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