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Synthesis and *In Vitro* Cytotoxicity of A Homologous Series of 5-Halosubstituted 1,3-Bis(ω-cyanoalkyl)uracil Analogues

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A homologous series of twenty, hitherto unreported, analogues of 5-halosubstituted 1,3-bis(ω-cyanoalkyl)uracil acyclic nucleosides were synthesized by the series of alkylation reactions of 5-halouracils with the corresponding chloroacetonitrile, chloropropionitrile, chlorobutyronitrile and 5-chlorovaleronitrile ($Cl-(CH_2)_n-CN$: n=1, 2, 3, 4) in anhydrous DMSO (or DMF)/K_2CO_3 (or NaH) under 75°C temperature. Antitumor activities for the synthesized compounds were determined against three cell lines (FM-3A cell, P-388 cell and U-938 cell lines). The compounds that exhibited moderate activity to significant activity, included **1a-b**, **2a-b**, **3a-c**, and **4a**, whose compounds were active against P-388, FM-3A and U-937 cell lines with the compounds **1a**, **1b**, and **2a**, showing significant antitumor activity (inhibitory concentrations (IC_{50}) ranged from 2.2 to 7.0 μg/ml). Their strucrure-activity relationship did not show any activity differences in their effective chain length (methyl, ethyl, propyl, butyl) in 1,3-bis(ω-cyanoalkyl) uracils.

Key words : 1,3-Bis(ω-cyanoalkyl)uracil, Acyclic Nucleoside, Antitumor Activity, Mouse mammary carcinoma (FM-3A), Mouse lymphoid neoplasma (P-388), Human histiocytic lymphoma (U-937). Inhibitory concentration (IC_{50})

INTRODUCTION

Since the discovery of 3'-azido-3'-deoxythymidine, AZT (Mitsuya, *et al.*, 1985) as a potent inhibitor of human immundeficiency virus type 1 (HIV-1) *in vitro* (Nasr, *et al.*, 1990), many nucleoside anologues in which the cyclic carbohydrate moiety was replaced by an acyclic side chain, have recently been reported to possess broad-spectrum antiviral activity in both cell culture systems and animal models (De Clereq, *et al.*, 1986). Although the cyclic nucleosides such as AZT (Fischl, *et al.*, 1987), DDI (Lambert, *et al.*, 1990) and DDC (Mitsuya, *et al.*, 1986) have been reported to be clinically useful to treat AIDS either alone or in combination, they suffer from serious toxicity, headaches, drug resistane and suppression of bone-marrow cell formation (Larder, *et al.*, 1989: Elion, *et al.*, 1977; Kametami, *et al.*, 1982). In order to discover more potent and less toxic agents as well as to meet the continuous challenge of drug resistance, various analogues were synthesized and evaluated against antiviral and antitumor activity (Chu, *et al.*, 1986; Schinazi, *et al.*, 1992; Kim, *et al.*, 1992, 1994 a-c; Marr, *et al.*, 1984; Urbina, *et al.*, 1991).

Therefore as part of our efforts to discover more useful antitumor agents, we prepared a number of 5-substituted pyrimidine homologues; 1, 3-bis (cyanomethyl)-5-substituted uracils (**1a-e**), 1, 3-bis(2-cyanoethyl)-5-substituted uracils (**2a-e**), 1, 3-bis(3-cyanopropyl)-5-substituted uracils (**3a-e**), and 1, 3-bis (4-cyanobutyl)-5-substituted uracils (**4a-e**) and evaluated for their *in vitro* cytotoxicities against three cell lines; mouse lymphoid neoplasma (P-388), human histiocytic lymphoma (U-937) and mouse mammary carcinoma (FM-3A) (Carmichael, *et al.*, 1987 ; Mosmann, 1983; Kim, *et al.*, 1994a-c).

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MATERIALS AND METHODS

Melting points were determined on electrothermal capillary melting point apparatus and are uncorrected. TLC was performed on glass plates coated with silica gel (silica gel 60 F_{254}) and compounds were visualized using an UV lamp. Proton magnetic resonance spectra were obtained with Varian EM-360A spectrophotometer and Varian Gemini 200 MHz (solution in dimethylsulfoxide-d_6 with tetramethylsilane as internal standard). Ultraviolet spectral data were measured with Hitachi 124 spectrometer. The organic sol-

Table I. Physicochemical Data for 1, 3-Bis(ω-cyanoalkyl)uracil Acyclic Nucleoside Analogues (**1a-e**, **2a-e**, **3a-e** and **4a-e**)

Comp. No.		m.p. (°C)	Recrystal. solvent	Yield (%)	IR (KBr) NMR (DMSO-d_6) ppm, δ cm^{-1}						UV(DMF) nm
	X				C≡N	5-H	6-H	$-NCH_2$	$-N'CH_2$	$-CH_2CN$	$-CH_2CH_2$	λ_{max}
1a	F	133-135	Ethanol	46	2254		8.3	4.9	3.4			274.8
b	Cl	149-151	Ethanol	43	2259		8.3	4.9	3.4			277.8
c	Br	175-177	Ethanol	45	2255		8.4	4.9	3.4			278.6
d	I	209-210	Ethanol	52	2254		8.4	4.9	3.4			282.4
e	H	101-102	Chloroform	39	2250	5.8	7.7	4.8	4.7			274.8
2a	F	139-140	Ethanol	46	2253		7.5	4.0	3.8	2.8		274.6
b	Cl	147-148	Ethanol	48	2253		7.9	4.0	3.8	2.8		276.8
c	Br	152-153	Ethanol	68	2251		7.6	4.3	4.0	2.8		279.6
d	I	147-149	Ethanol	56	2249		8.3	4.0	2.9	2.8		296.0
e	H	125-127	Ethanol	31	2250	5.7	7.6	3.9	3.8	2.9		274.4
3a	F	oily	----------	56	2251		7.4	4.0	3.9	2.5	2.0	272.8
b	Cl	82-85	Ethanol	48	2248		7.4	4.1	3.9	2.4	2.0	279.4
c	Br	83-85	Ethanol	61	2247		7.6	4.1	3.9	2.4	2.0	281.2
d	I	93-95	Ethanol	58	2245		7.6	4.1	3.9	2.4	2.0	285.8
e	H	oily	----------	42	2250	5.8	7.2	3.9	3.8	2.5	2.1	276.6
4a	F	oily	----------	48	2252		7.4	4.0	3.9	2.3	1.7	271.6
b	Cl	52-54	Ethanol	57	2248		7.4	4.0	3.8	2.4	1.8	280.0
c	Br	60-61	Ethanol	52	2246		7.5	4.0	3.8	2.4	1.7	282.4
d	I	72-74	Ethanol	62	2245		7.6	4.0	3.8	2.4	1.8	288.0
e	H	47-49	Ethanol	41	2250	5.8	7.2	4.0	3.8	2.4	1.8	267.6

vents and chemicals were obtained from the commercial and purified by the appropriate methods before use. Pertinent data for sythesized compounds (**1a-e**, **2a-e**, **3a-e** and **4a-e**) are listed in Table 1.

General procedure for the synthesis of 1, 3-bis(ω-cyanoalkyl) uracils (1a-e, 2a-e, 3a-e and 4a-e)

To a stirred solution of uracil (4.06 mmol) in anhydrous DMF (or DMSO) (40 ml) was added K_2CO_3 (4.06 mmol) and heated to 75°C. The heterogeneous reaction mixture was continously heated by adding ω-cyanoalkyl chloride (4.17 mmol) in small portions for 7 hours and the solvent was removed by evaporation to give a solid. Crystallization from an appropriate solvent afforded an analytically pure solid. The uncrystallized oily residues were applied to a column packed with silica gel and the column was eluted with hexane-ethyl acetate (20 : 1, v/v) (Table I).

Evaluation of antitumor activity.

The antitumor effect of the synthesized compounds was determined by the modified methods (Mosmann, *et al.*, 1983 ; Carmichael, *et al.*, 1987) (Table II).

MTT-Microculture Tetrazolium Assay.

The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co., St. Louis, M. O.) by the mitochondrial dehydrogenase of vial cells to a blue water-nonsoluble formazan crystal product which can be measured spectrophotometrically (Mosmann, *et al.*, 1983 ; Carmichael, *et al.*, 1987 ; Kim, *et al.*, 1994a-c). Following appropriate incubation of cells (P-388, FM-3A and U-937 cells) in the presence or absence of synthesized compounds, the [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, M.O.) was added to each well and incubated at 37°C for a further 4 hours before processing as decribed below.

For cell growth, serially increasing cell numbers were plated in different columns across 96-well microtiter plates. Well growing cells were harvested, counted and inoculated at the concentrations of 2×10^4 cells/ml into 96-well microtiter plates. After 24 hours, synthesized compounds (**1a-e**, **2a-e**, **3a-e** and **4a-e**) were applied to triplicate culture wells and the cultures were incubated at 37°C for 3 days. Following this incubation, 2 μl of MTT solution (5 mg/ml in phosphate buffer solution; KCl 0.2 g, KH_2PO_4 0.2 g, NaCl 8.0 g, Na_2HPO_4 1.15 g, $MgCl_2$ 0.101 g/l, pH=7.4) was added to microculture wells. After 4 hours incubation at 37°C, the supernatant was removed from each well and 100 μl of 100% DMSO was added to solubilize the formazan crystals which were formed by the celluar reduction of MTT. After thorough mixing with mechanical plate mixer, absorbance spectra was read on ELISA Processor II microplate Reader (Behering Co.) at a wavelength of 570 nm and a reference wavelength of 650 nm (absorbance peak for DMSO). All measurements were carried out in tri-

Table II. IC_{50} Values for 1, 3-Bis(ω-cyanoalkyl)uracil Acyclic Nucleoside Analogues (**1a-e**, **2a-e**, **3a-e** and **4a-e**)

comp. no.	IC_{50} (μg/ml)[a]		
	P-388[b]	U-937[c]	FM-3A[d]
1 a	7.0	5.6	5
b	3.6	4.4	2.2
c	26	52	24
d	52	59	10
e	>100	82	20
2 a	3.6	3.1	3.1
b	35	44	5
c	>100	>100	32
d	>100	>100	36
e	>100	>100	83
3 a	33	65	16
b	>100	44	7.4
c	39	50	6.4
d	>100	59	80
e	>100	50	70
4 a	40	61	7.2
b	>100	>100	22.5
c	74	>100	44
d	>100	58	35
e	>100	>100	53

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: Mouse lymphoid neoplasma.
c: Human histiocytic lymphoma cell.
d: Mouse mammary carcinoma cell.

plicate. There was good reproducibility between replicate wells with standard errors $\leq$ +10% (Carmichael, *et al.*, 1987) (Tabble II).

RESULTS AND DISCUSSION

A number of the acyclic nucleoside homologues; 1, 3-bis(cyanomethyl)-5-halouracils (**1a-e**), 1, 3-bis (cyanoethyl)-5-halouracils (**2a-e**), 1, 3-bis(3-cyanopropyl)-5-halouracils (**3a-e**) and 1, 3-bis (4-cyanobutyl)-5-halouracils (**4a-e**), lacking the D-ribose sugar part (Schaeffer, *et al.*, 1978; Kelley, *et al.*, 1981), were prepared using the standard synthetic route. Alkylation of 5-halosubstituted uracils with the corresponding chloroacetonitrile, chloropropionitrile, chlorobutyronitrile and 5-chlorovaleronitrile in anhydrous DMSO (or DMF) / K_2CO_3 (or NaH) under 75°C temperature afforded moderate yields of 1, 3-bis(ω-cyanoalkyl)-5-substituted uracils (Table I). A homologous series of the bis-alkylated products, **1a-e**, **2a-e**, **3a-e** and **4a-e**, were purified on silica gel and the structure of the synthesized compounds were identified by the FT-IR ^{1}H-NMR, UV and some compounds were identified with mass spectra.

Twenty, hitherto unreported, compounds of 1, 3-bis (ω-cyanoalkyl)uracil homologues (**1a-e**, **2a-e**, **3a-e** and **4a-e**) were evaluated for antitumor efficacy against the following cell lines; a) mouse mammary carcinoma (FM-3A), b) mouse lymphoid neoplasma (P-388) and c) human histiocytic lymphoma (U-937). The cytotoxicity of the synthesized compounds against three cell lines measured as IC_{50} values, are given in Table II. The compounds that exhibited moderate activity to significant activity, included **1a-b**, **2a-b**, **3a-c** and **4a** whose compounds were active against P-388, FM-3A and U-937 cell lines with the compounds **1a**, **1b**, and **2a** showing significant antitumor activity [inhibitory concentrations (IC_{50}) ranged from 2.2 to 7.0 μg/ml]. Rest of the 5-substituted analogues did not show any antitumor activity, and their strucrure-activity relationship did not show any activity differences in their effective chain length (methyl, ethyl, propyl, butyl) between pyrimidine nucleic acid base and ω-cyanoalkyl moiety.

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