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5-Substituted Pyrimidine Acyclic Nucleoside Analogues 1-Cyanomethyl- and 1-(4-Cyanobutyl)-5-substituted Uracils as Candidate Antitumor Agents

Jack C. Kim[1], Eun-Soo Dong[1], Jin Il Park[1], Sang-Duk Bae[1] and Seon-Hee Kim[2]

[1]*Department of Chemistry, College of Natural Science,* [2]*Department of Biochemistry, College of Medicine, Pusan National University, Pusan 609-735, Korea*

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A number of 5-substituted pyrimidine acyclic nucleosides were synthesized and tested for in vitro cytotoxicity against four cell lines (J-82 cell, P-388 cell, FM-3A cell and U-938 cell lines). Synthesis of 1-cyanomethyl-5-substituted pyrimidines (**1a-e**) and 1-(4-cyanobutyl)-5-substituted pyrimidines (**2a-e**) was acomplished from the series of alkylation reactions of 5-substituted uracils with the corresponding chloroacetonitrile and 5-chlorovaleronitile in DMSO under 50℃ temperature. These 5-substituted pyrimidine acylic nucleosides (**1a-e** and **2a-e**) exhibited moderate to significant activity against four cell lines.

Key words: Acyclic nucleoside analogues, 1-Cyanomethyl-5-substituted pyrimidines, 1-(4-Cyanobutyl)-5-substituted pyrimidines, Antitumor activity, Human bladder carcinoma cell(J-82), Mouse mammary carcinoma (FM-3A), Mouse lymphoid neoplasma (P-388), Human histiocytic lymphoma (U-937), IC_{50}

INTRODUCTION

The aliphatic nucleoside analogues, 9-[2-(hydroxyethoxy)methyl]guanine, and 9-(2,3-dihydroxypropyl)adenine, in which the cyclic carbohydrate moiety was replaced by an acylic side chain, have recently been reported to possess broad-spectrum antiviral activity in both cell culture systems and animal models (De Clercq *et al.*, 1986; Ellion *et al.*, 1977; Schaeffer *et al.*, 1978). In studies aimed at delineating the structural requirements that underlie the marked antiviral activity of the above compounds, it was reasoned that various aliphatic nucleoside analogues might also possess antiviral and antitumor potentials (Chu *et al.*, 1986; Kametani *et al.*, 1982; Kelley *et al.*, 1981; Kim *et al.*, 1992; 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 acyclic 5-substituted pyrimidine nucleoside analogues, 1-cyanomethyl-5-substituted uracils (**1a-e**) and 1-(4-cyanobutyl)-5-substituted uracils (**2a-e**), and evaluated for their in vitro cytotoxicities against four cell lines (J-82 cell, P-388 cell, FM-3A cell and U-938 cell lines) (Carmichael *et al.*, 1987; Kim *et al.*, 1994a,b; Mosmann, 1983).

Correspondence to: Jack C. Kim, Department of Chemistry, Pusan National University, Pusan, Korea

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

General Procedure for the Preparation of 1-Cyanomethyl-5-Substituted Uracils (1a-e) and 1-(4-Cyanobutyl)-5-Substituted Uracils (2a-e)

A solution of uracil (2.56 mmol) in DMSO (15 ml) was dissolved at 50℃ temperature and treated with

Table I. Physicochemical Data for 1-Cyanomethyl-5-substituted Uracils (**1a-e**) and 1-(4-Cyanobutyl)-5-substituted Uracils (**2a-e**)

Comp. Num.	n	X	m.p. (℃)	Recryst. Sol.	Yield (%)	IR(KBr) C≡N	NMR(DMSO-d_6) ppm 5-H	6-H	-N-CH_2	-CH_2-N	CH_2CH_2-	UV(DMF)nm λmax
1	a	F	288-289	Ethanol	23	2252 cm^{-1}		δ7.5	δ4.0			274.7
1	b	Cl	292-293	Ethanol	27	2250 cm^{-1}		δ7.8	δ4.1			276.6
1	c	Br	294-295	Ethanol	29	2249 cn^{-1}		δ7.8	δ4.1			279.4
1	d	I	290-292	Ethanol	24	2249 cm^{-1}		δ7.9	δ4.1			282.8
1	e	H	287-289	Ethanol	24	2250 cm^{-1}	δ5.4	δ7.6	δ4.9			274.6
4	a	F	129-131	Ethanol	47	2257 cm^{-1}		δ8.1	δ3.6	δ2.5	δ1.6	274.6
4	b	Cl	165-166	Ethanol	41	2252 cm^{-1}		δ8.2	δ3.7	δ2.5	δ1.6	277.4
4	c	Br	164-166	Ethanol	51	2251 cm^{-1}		δ8.2	δ3.7	δ2.5	δ1.6	282.4
4	d	I	162-164	Ethanol	33	2243 cm^{-1}		δ8.2	δ3.7	δ2.5	δ1.6	287.6
4	e	H	85-87	Ethanol	53	2250 cm^{-1}	δ5.7	δ7.2	δ3.8	δ2.4	δ1.7	277.2

Table II. IC_{50} Values for 1-Cyanomethyl5-substituted Uracils (**1a-e**) and 1-(4-Cyanobutyl)-5-substituted Uracils (**2a-e**)

Comp.	IC_{50} (μg/ml)[a] J-82[b]	P-388/s[c]	FM-3A/s[d]	U-937/s[e]
1a	>480.4	7.0	5.0	5.6
1b	13.8	3.6	2.2	4.4
1c	>371.7	26	24	53
1d	333.3	52	10	59
1e	>525.3	>100	20	82
2a	>473.4	>100	–	>100
2b	281.1	>100	–	>100
2c	271.9	>100	74	>100
2d	>313.4	>100	>100	>100
2e	543.4	45	45	70

[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 bladder carcinoma cell. [c]Mouse leukemia cell. [d]Mouse mammary carcinoma cell. [e]Human histioticytic lymphoma cell

K_2CO_3 (2.56 mmol). The reaction mixture was stirred by adding 4-chlorobutyronitrile (2.13 mmol) in small portions during 3-4 hours period. The reaction mixture was evaporated to give 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 fractions containing the **1a-e** and **2a-e** were collected first, and concentrated to a syrup which was crystallized from an appropriate solvent (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.*, 1994). Following appropriate incubation of cells (J-82, 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℃ 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 cell 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** and **2a-e**) were applied to triplicate culture wells and the cultures were incubated at 37℃ for 3 days. Following this incubation, 2 μl of MTT solution (5 mg/ml in phosphate buffer solution; KCl 0.2g, 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℃, 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 triplicate. There was good reproducibility between replicate wells with standard erors ≤+10% (Carmichael *et al.*, 1987).

RESULTS AND DISCUSSION

A number of 5-substituted pyrimidine acyclic nucleoside analogues; 1-cyanomethyl-5-fluorouracil (**1a**), 1-

cyanomethyl-5-chlorouracil (**1b**), 1-cyanomethyl-5-bromouracil (**1c**), 1-cyanomethyl-5-iodouracil (**1d**) 1-cyanomethyluracil (**1e**), 1-(4-cyanobutyl)-5-fluorouracil (**2a**), 1-(4-cyanobutyl)-5-chlorouracil (**2b**), 1-(4-cyanobutyl)-5-bromouracil (**2c**), 1-(4-cyanobutyl)-5-iodouracil (**2d**) and 1-(4-cyanobutyl)uracil (**2e**), lacking the D-ribose sugar part, were preapared using the standard synthetic route. Alkylation of 5-substituted uracils with the corresponding chloroacetonitrile and 5-chlorovaleronitrile (K_2CO_3, DMSO) afforded moderate yields of 1-cyanomethyl-5-substituted uracils (**1a-e**) and 1-(4-cyanobutyl)-5-substituted uracils (**2a-e**) (Table I). A homologous series of the alkylated products, **1a-e** and **2a-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.

Ten, hitherto unreported, compounds of acyclic nucleoside homologues, **1a-e** and **2a-e** were evaluated for antitumor efficacy against the following cell lines; a) human bladder carcinoma cell (J-82), b) mouse leukemia cell (P-388/s), c) mouse mammary carcinoma (FM-3A/s) and d) human histiocytic lymphoma (U-937 /s). The cytotoxicity of the synthesized compounds (**1a-e** and **2a-e**) against four cell lines measured as IC_{50} values are given in Table II.

The compounds that exhibited moderate to significant activity, included **1a**, **1b**, **1c** and **1d** whose compounds were active against P-388/s, FM-3A/s and U-937/s cell lines with the compound **1a** and **1b** showing significant antitumor activity (inhibitory concentration (IC_{50}) ranged from 2.2 to 5.6 μg/ml). Rest of the 5-substituted acyclic nucleoside analogues did not show any antitumor activity.

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REFERENCES CITED

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., Mitchell, J. B., Evaluation of a Tetrazolium-based Semiautomated Colorimetric Assay; Assessment of Chemosensitivity Testing. *Canceer Res.*, 47, 936 (1987).

Chu, C. K., Cutter, S. J., Chemistry and Antiviral Activities of Acyclonucleosides. *J. Heterocycl. Chem.*, 23, 289-319 (1986).

De Clercq, E., Walker, R. T., Progress in Medicinal Chemistry; Ellis, G.P., West, G. B., Eds., Elsevier; New York, 1986; Vol. 23, Chapter 5.

Elion, G. B., Furman, P. A., Fyfe, J. A., DeMiranda, P., Beauchamp, L., Schaeffer, H. J., Selectivity of Action of an Antiherpetic Agent, 9-(2-Hydroxyethoxymethyl) Guanine. *Proc. Natl. Acad. Sci. U.S.A.*, 74, 5716-5720 (1977).

Kametani, T., Kigasawa, K., Hiiragi, M., Wakisawa, K., Nakazato, K., Ichikawa, K., Fukawa, K., Irino, O., Nishimura, N., Okada, T., Studies on the Synthesis of Chemotherapeutics. 12. Synthesis and Antitumor Activity of N-phthalidyl-5-Fluorouracil Derivatives. *J. Med. Chem.*, 25, 1219-1222 (1982).

Kelley, J. A., Kelsey, J. E., Hall, W. R., Krochmal, M. P., Schaeffer, H. J., Pyrimidine Acyclic Nucleosides, 1-[2-(hydroxyethoxy)methyl]pyrimidines as Candidate Antivirals. *J. Med. Chem.*, 24, 753-756 (1981).

Kim, J. C., Bae, S. S., Kim, S. H., Synthesis and *In Vitro* Cytotoxicity of a Homologues Series of 9-[ω-(N'-Methyl-N'-Nitrosoureido)alkyl] purines. *Korean J. Med. Chem.*, 4, 66-72 (1994a).

Kim, J. C., Dong, E. S., Kim, S. H., Kim, S. H., Synthesis and Evaluation of Antitumor Activity of a Homologous Series of 1-(ω-Cyanoalkyl)- and 1,3-Bis(ω-Cyanoalkyl) Uracil Nucleoside Analogouses. *Arch. Pharm. Res.*, 17, 135-138 (1994b).

Kim, J. C., Lee, Y. H., Synthesis and Evaluation of Uracil-6-carboxaldehyde Schiff Bases as Potential Antitumor Agents. *Korean J. Med. Chem.*, 2, 64-69 (1992).

Marr, I. I., Berens, R. L., Cohn, N. K., Nelson, D. J., Klein, R., Biological Action of Inosine Analogs in Leishmania and Trypanosoma spp. *Antimicrob. Agents Chemother.*, 25, 292-295 (1984).

Mosmann, T., Rapid Colorimetric Assay for Celluar growth and Survival Application to Prodiferation and Cytotoxicity Assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Schaeffer, H. J., Beauchamp, L., Miranda, P., Elion, G. B., Bauer D. J., Collins, P., 9-(2-Hydroxyethoxymethyl) guanine Activity Against Viruses of the Herpes Group. *Nature*, 272, 583-585 (1978).

Urbina, J. A., Lazardi, K., Aguirre, M., Piras, M. M., Piras, R., Antiproliferative Effects and Mechanism of Action of ICI 195, 739 a novel Bis-triazole Derivative. *Antimicrob. Agents Chmother.*, 35, 730-735 (1991).