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Synthesis and Evaluation of Antitumor Activity of a Homologous Series of 1-(ω-Cyanoalkyl)-and 1,3-Bis (ω-Cyanoalkyl)uracil Nucleoside Analogues

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Acyclonucleoside homologues of 1-(ω-cyanoalkyl)-and 1,3-bis (ω-cyanoalkyl) uracils were synthesized by the series of alkylation reactions of uracil with the ω-chloroalkyl nitrile ($Cl-(CH_2)_n-CN$; n=1, 2, 3, 4) in DMSO under 50~75℃ temperature. The 1-(ω-cyanoalkyl)-and 1,3-bis(ω-cyanoalkyl) uracils were separated either by the fractional crystallization or column chromatography. The antitumor activities for these synthesized compounds were determined against four cell lines (J-82 cell, P388 cell, FM-3A cell and U-937 cell lines). These compounds failed to exhibit any significant antitumor activity.

Key words: 1-(ω-Cyanoalkyl)uracil, 1,3-bis(ω-Cyanoalkyl)uracil, Acyclonucleoside homologues, Antitumor activity, Human bladder carcinoma cell (J-82), Mouse mammary carcinoma (FM-3A), Mouse lymphoid neoplasma (P-388), Human histiocyttic lymphoma (U-937), IC_{50}

INTRODUCTION

Since the discovery of AZT (Mitsuya *et al.*, 1985) in 1985, a number of nucleosides have been identified as potent and promising anti-HIV agents (Nasr *et al.*, 1990). AZT (Fischl *et al.* 1987), DDI (Hambert *et al.*, 1990), and DDC (Mitsuya *et al.*, 1986) have been approved for the treatment of AIDS and antitumor related complex. Although AZT, DDI and DDC have been reported to be clinically useful to treat AIDS and cancer patients either alone or in combination, side effects, toxicities and drug resistance may limit their usefulness (Larder *et al.*, 1989). In order to discover more potent and less toxic compounds, a number of nucleosides were synthesized and evaluated against HIV and antitumor activity in vitro. Among these, dioxolane (Schinazi *et al.*, 1992; Norbeck *et al.*, 1989) and oxathiolane (Coates *et al.*, 1992) nucleosides are unique in that the classical carbohydrate moieties of nucleosides are replaced with dioxolane and oxathiolane ring. A series of modifications in the sugar part of nucleosides (Schaeffer *et al.*, 1978; Elion *et al.*, 1977) have led to the development of several nucleoside analogues with antiviral and antitumor properties. Well-known examples are acyclic nucleoside analogues (eg, ACV) (Marr *et al.*, 1984), pentose-fluorinated nucleosides (eg, FMAU, FIAU) (Urbina *et al.*, 1991), carbocyclic nucleosides (eg, carbovir) (Chu *et al.*, 1986), nucleosides with a four-membered ring (eg, oxetanocin) (De Clercq *et al.*, 1986), and phosphonylated analogues of acyclic nucleosides (eg, PMEA) (Kametani *et al.*, 1982).

It was reasoned that acyclic nucleoside analogues of biologically important pyrimidine nucleosides might also possess interesting biological activity (Kim *et al.*, 1992). Therefore as part of our efforts to discover useful antitumor agents, we synthesized a number of the acyclic nucleoside homologues, 1-(ω-cyanoalkyl)uracils, **1a-d** and 1,3-bis(ω-cyanoalkyl)uracils, **2a-d**, lacking the normal glycosidic part.

MATERIALS AND METHODS

Melting points were determined on electrothermal capillary melting point apparatus and are uncorrected. TLC was performed on glass plates coated with aluminium oxide (silica gel 60 F254) and compounds were visualized using an UV lamp. Proton magnetic resonance spectra were obtained with Varian EM-360A spectrophotometer (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

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Table I. 1-(ω-Cyanoalkyl)uracils (**1a-d**)

Comp	n	mp	Recryst	yield	IR(KBr)	NMR (DMSO-d_6) (δ)					UV (DMF)
No		(℃)	solvent	(%)	υ_{CN} (cm^{-1})	5-H	6-H	N-CH_2	CH_2-CN	CH_2CH_2	α_{max} (nm)
a	1	287-289	EtOH	24	2250	5.4	7.6	4.9			273.6
b	2	226-228	EtOH	75	2250	5.6	7.6	3.9	2.9		274.0
c	3	150-152	EtOH	45	2250	5.9	7.1	3.6	2.5	2.1	274.2
d	4	85- 87	EtOH	53	2250	5.7	7.2	3.8	2.4	1.7	227.2

Table II. 1,3-Bis(ω-Cyanoalkyl)uracils (**2a-d**)

Comp	n	mp	Recryst	yield	IR(KBr)	NMR (DMSO-d_6) (δ)						UV (DMF)
No		(℃)	solvent	(%)	υ_{CN} (cm^{-1})	5-H	6-H	N^1-CH_2	N^3-CH_2	CH_2-CN	CH_2CH_2	α_{max} (nm)
a	1	101-102	EtOH	39	2250	5.8	7.7	4.8	4.9			274.8
b	2	125-127	EtOH	31	2250	5.7	7.6	3.9	2.9			274.4
c	3	oily	—	42	2250	5.8	7.2	3.9	3.8	2.1		276.6
d	4	47-49	EtOH	41	2250	5.8	7.2	4.0	4.0	2.4	1.8	267.6

from commercial and purified by the appropriate methods before use. Pertinent data for synthesized compounds (**1a-d** and **2a-d**) are listed in Table I and II.

General Procedure for the preparation of 1-(ω-cyanoalkyl)uracils (1a-d, Table I)

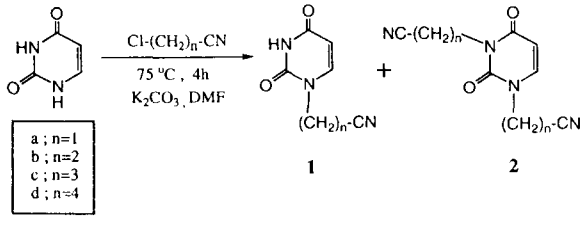
A solution of uracil (3.84 mmol) in DMSO (20 ml) was dissolved at 60℃ temperature and treated with K_2CO_3 (3.84 mmol). The reaction mixture was stirred by adding 4-chlorobutyronitrile (3.19 mmol) in small portions during 3~4 hours period. The reaction mixture was evaporated to give oily residues, which were applied to a column packed with silica gel and the column was eluted with hexane-ethylacetate (20 : 1, v/v). The fractions containing the **1a-d** were collected first, and concentrated to a sirup which was crystallized from an appropriate solvent. Further elution gave the **2a-d**.

General procedure for the synthesis of 1, 3-Bis(ω-cyanoalkyl)uracils (Table II, 2a-d)

To a stirred solution of uracil (3.41 mmol) in DMF (30 ml) was added K_2CO_3 (3.41 mmol) and heated at 65℃. The heterogeneous reaction mixture was continously heated by adding 4-chlorobutyronitrile (3.50 mmol) in small portions for 6 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-ethylacetate (20 : 1, v/v).

Evaluation of Antitumor Activity

The antitumor effect of the synthesized compounds



Scheme 1

was determined by the modified methods (Mosmann *et al.*, 1983; Carmichael *et al.*, 1987).

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). Following appropriate incubation of cells (J-82, P-388, FM-3A and U-937 cells) in the presence or absence of synthesized compounds, [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 described 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-d**, and **2a-d**) were applied to triplicate culture wells and the cultures were incubated at 37℃ for 3 days. Following this incubation, 20 μl of MTT solution (5 mg/ml in phosphate buffer solu-

tion; 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/ml, 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 cellular reduction of MTT. After thorough mixing with a mechanical plate mixer, absorbance spectra was read on on ELISA Processor II microplate Reader (Behring Co.) at a wavelengh 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 $\leq +10\%$ (Carmichael *et al.*, 1987).

RESULTS AND DISCUSSION

A number of the acyclic nucleoside homologues; 1-(cyanomethyl)uracil, **1a**, 1-(2-cyanoethyl)uracil, **1b**, 1-(3-cyanopropyl) uracil, **1c**, 1-(4-cyanobutyl)uracil. **1d**, 1, 3-bis-(cyanomethyl)uracil, **2a**, 1,3-bis(2-cyanoethyl) urail, **2b**, 1,3-bis(3-cyanopropyl)uracil, **2c**, 1,3-bis(4-cyanobutyl)uracil, **3d**, lacking the D-ribose sugar part, were prepared using the standard synthetic route outlined in Scheme 1. Alkylation of uracil with ω-chloroalkyl nitrile (K_2CO_3, DMSO) afforded moderate yields of the 1-(ω-cyanoalkyl)uracils, **1a-d** (Table I), and the 1,3-bis(ω-cyanoalkyl)uracils, **2a-d** were obtained by the same method under the more vigorous conditions of 50~70℃ temperature. A homologous series of the alkylated products, **1a-d** and **2a-d** were purified on silica gel and the structure of the synthesized compounds were identified by the FT-1R, ^{1}H-NMR, UV and some compounds were identified with mass spectra.

All the acyclic nucleoside homologues, **1a-d** and **2a-d** 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); d) human histiocytic lymphoma (U-937/s), and none of our synthesized compounds showed any significant antitumor activity against the above four cell lines. The observations that the acyclic nucleoside homologues, **1a-d** and **2a-d** are devoid of antitumor activity, indicate that a design of new compounds should be made for a further examination.

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