SYNTHESIS AND ANTITUMOR ACTIVITY OF N-NITROSOUREIDO DERIVATIVES OF KANOSAMINE

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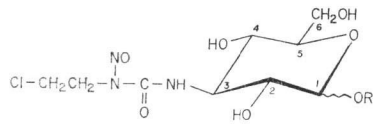
Structure-activity studies with the non-myelosuppressive methylnitrosourea, streptozotocin (SZ), and its 2-chloroethyl analogue, chlorozotocin (DCNU), have suggested that bone marrow toxicity could be reduced by attachment of the cytotoxic group to the carbon-2 position of glucose.[1,2] It has also been reported that DCNU produced no significant depression in normal bone marrow DNA synthesis, in contrast to a sustained >90% inhibition in the L1210 ascites cell DNA synthesis[3].

Many reports have recently appeared on the other water-soluble sugar analogues such as GANU[4], 2MCαG and 6MCαG[5] having antileukemic properties. We wish to report here the preparation and antitumor activity of a new sugar analogue, 1-(2-chloroethyl)-3-(D-glucos-3-yl)-1-nitrosourea (**I**, KCNU) which is a derivative of kanosamine (3-amino-3-deoxy-D-glucose), and its methyl glycoside KCNU-M (**II**).

Synthesis of KCNU [NSC 319735D] (**I**) was carried out according to NAKAO's simple, one-step approach for the synthesis of a series of N-(2-chloroethyl)-N-nitrosourea[6]. Reaction of D-kanosamine, which was obtained by the fermentation of *Bacillus* sp.[7,8], a strain PB-2, with *o*- or *p*-nitrophenyl N-(2-chloroethyl)-N-nitrosocarbamate at room temperature gave directly the desired product in good yield: **I**, $C_9H_{16}N_3O_7Cl$*, mp 104~105°C, $[\alpha]_D^{20}$ +23° (*c* 1, methanol); ir (KBr) 1730, 1550 (amide carbonyl), and 1500 (N-NO) cm^{-1}; λ_{max}^{MeOH} 231, 383, 399 and 417 nm ($E_{1cm}^{1\%}$ 170, 1.5, 2.2 and 1.9); and cmr (in D_2O): 40.48 (N-CH_2), 42.14 (CH_2-Cl), 58.33 (α-C3), 59.79 (β-C3), 61.50, 61.69 (α and β-C6), 68.62, 68.77, 70.52, 72.67, 73.15, 77.93 (α and β-C2, 4 and 5), 92.56 (α-Cl), 97.44 (β-Cl) and 167.96 (N-CO-N) ppm. Tetra-O-acetyl ester of **I**; $C_{17}H_{24}N_3O_{11}Cl$*, mp 113~114°C, $[\alpha]_D^{20}$ +39° (*c* 1, methanol); and mass spec.: *m/z* 481 (M^+). By employing the same procedure, the reaction of methyl D-kanosaminide with *p*-nitrophenyl N-(2-chloroethyl)-N-nitrosocarbamate afforded 1-(2-chloroethyl)-3-(1-O-methyl-D-glucos-3-yl)-1-nitrosourea (**II**, KCNU-M [NSC 319736D]): $C_{10}H_{18}N_3O_7Cl$*, mp 108~110°C, $[\alpha]_D^{20}$ +61° (*c* 1, methanol); ir (KBr) 1730, 1545 (amide carbonyl) and 1500 (N-NO); and mass spec.: *m/z* 327 (M^+).



I : R = H
II : R = CH_3

The two compounds are presently undergoing laboratory studies. To determine the *in vivo* antitumor activity, the tumor model of the ip murine L1210 lymphoid leukemia, B16 melanocarcinoma and Colon 26 xenograft were employed for the evaluation. In an ip test it is necessary to have, in general, a minimal increase in survival of treated animals over controls resulting in a T/C ≧125% for L1210 and for B16, and ≧130% for Colon 26 according to NCI antitumor test protocol[9]. Aqueous saline solution was used as a drug vehicle. Treatment schedule was employed as shown in the legends of Tables. No death of the host animals in all experiments was observed on the toxicity day 4 after day of first drug injection. Intraperitoneal antitumor data are given in Tables 1 ~3.

Tables list those compounds with outstanding activity in the employed systems. More significant activity was obtained with KCNU-M (**II**) than KCNU (**I**) in all treatments. Multiple cures, defined as survivors either on day 40 in the L1210 system, or on day 60 in the Colon 26 system, were observed for both compounds. Compound **II** was especially active producing multiple cures on day 40 in the L1210 system at the lower dose level.

* Microanalyses for CHNOCl agreed with the assigned formula.

Table 1. Intraperitoneal lymphoid leukemia L1210 activity.

Compound	Dose, mg/kg/day	MST*, days	T/C, %	Survivors/total at day 40
I	90.0	>40.0	>556	5/5
	60.0	>40.0	>556	5/5
	30.0	>40.0	>556	5/5
	10.0	17.2	139	1/5
II	60.0	14.5	101	1/5
	30.0	>40.0	>556	5/5
	10.0	31.2	433	3/5

Leukemia L1210 cells (10^5) were intraperitoneally inoculated into BD_2F_1 mouse. The drugs were intraperitoneally injected by single dose.
* Median survival time.

Table 2. Intraperitoneal B16 melanocarcinoma activity.

Compound	Dose, mg/kg day	MST*, days	T/C, %	Survivors/total at day 60
I	64.00	>60.0	>275	5/10
	32.00	35.0	160	0/10
	16.00	36.3	166	0/10
	8.00	27.1	124	0/10
II	18.00	32.0	146	2/10
	9.00	50.0	229	3/10
	4.50	44.3	203	0/10
	2.25	34.3	157	0/10

Tumor homogenate of B16 melanoma was diluted 10 times and inoculated intraperitoneally into $B_6C_3F_1$ mouse (female). The drugs were intraperitoneally injected by the chromic (QD1-9) treatment schedule.
* Median survival time.

Table 3. Intraperitoneal Colon 26 activity.

Compound	Dose, mg/kg/day	MST*, days	T/C, %	Survivors/total at day 60
I	128.0	>60.0	>495	10/10
	64.0	>60.0	>495	10/10
	32.0	>60.0	>495	6/10
	16.0	15.7	129	1/10
II	36.0	>60.0	>495	7/10
	18.0	>60.0	>495	10/10
	9.0	>60.0	>495	9/10
	4.5	20.0	165	1/10

Tumor homogenate of Colon 26 xenograft was diluted one hundred times and inoculated intraperitoneally into CD_2F_1 mouse (female). The drugs were intraperitoneally injected by Q4D treatment schedule (2 injections).
* Median survival time.

The ip acute toxicity value (LD_{50}) of both **I** and **II** was 150 mgs/kg in ICR/JCL female mice, respectively.

It has been reported that SZ and DCNU have a hyperglycemic effect[5,10,11] probably causing diabetes by affecting the pancreatic islet β cells of LANGERHANS of rodents and higher animal species. The former compounds has been used effectively to cause experimental diabetes in the mouse[12]. It is under investigation whether our compounds have such a diabetogenic effect in mice.

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