STRUCTURE-ANTITUMOR ACTIVITY RELATIONSHIP OF PURIN-6-YL ALKYL DISULFIDES*

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Antitumor activity and toxicity to host of newly synthesized disulfide derivatives of 6-mercaptapurine (6-MP) and 6-thioguanine (6-TG) were examined in murine ascites sarcoma-180 system (total packed cell volume method) by parenteral administration. The compounds tested were 6-alkyl disulfides (carbon number of alkyl group: 2, 3, 4, 5, 6, 7, 8, 10, and 14), 6-branched-alkyl disulfides (iso-propyl, *sec*-butyl, and *tert*-butyl), and 6-aralkyl disulfide (naphthyl). Most disulfide derivatives of 6-MP and 6-TG showed higher antitumor activity (lower ED₅₀) and higher toxicity to host (lower LD₅₀) than parent compounds, but ratios of increase in activity and toxicity were different with each other. The compounds with higher chemotherapeutic index (LD₅₀/ED₅₀) than parent compounds were α -naphthyl (8.7) disulfide in a series of 6-MP (7.5) derivatives; and *sec*-butyl (27), *tert*-butyl (24), octyl (23), decyl (26), and α -naphthyl (28) disulfides in a series of 6-TG (14) derivatives. These 6-TG derivatives were promising for antitumor agents.

Keywords—alkyl disulfides of 6-MP; alkyl disulfides of 6-TG; antitumor activity; ascites sarcoma-180; 6-mercaptapurine derivatives; 6-thioguanine derivatives.

INTRODUCTION

Early studies have shown that the main degradation pathway of 6-mercaptapurine (6-MP) and 6-thioguanine (6-TG) is oxidation of mercapto group at 6-position. To protect the mercapto group from oxidation, many 6-alkylthio derivatives of purine in which hydrogen atom of mercapto group was substituted with alkyl or aralkyl group were synthesized by Clarke *et al.*¹⁾ and Skipper *et al.*²⁾ Chemically the bond between alkyl group and sulfur is stable and it is relatively difficult to split and liberate the active parent compounds. Among them, 6-(1-methyl-4-nitro-5-imidazolyl)-thiopurine (Imuran) and -thioguanine are most effective against experimental tumors because the bond between the nitroimidazolyl group and sulfur is relatively labile owing to the electron-attracting nitro-

imidazolyl group.³⁾ As another comparable attempt, symmetrical disulfides of 6-MP, 6-TG, and their ribonucleosides were synthesized by Doerr *et al.*⁴⁾ and several assymmetrical disulfides of 6-MP and 6-TG with alkyl disulfide group at 6-position were made by us⁵⁾ and Ootsu *et al.*⁶⁾ These disulfide derivatives show somewhat higher antitumor activity than parent compounds. Since disulfide bond is more labile than alkylthio bond to reducing agents, it is expected that disulfide derivatives release the active parent compound more easily than alkylthio derivatives *in vivo*.

In the present paper, antitumor activity of a series of newly synthesized disulfide derivatives of 6-MP and 6-TG was investigated systematically to find the relationship between chemotherapeutic activity and chemical structure.

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MATERIALS AND METHODS

Compounds — 6-MP and 6-TG were purchased from Sigma Co. (U. S. A.). 6-Disulfide de-

rivatives of 6-MP and 6-TG were synthesized by the method reported previously.⁵⁾ Chemical structure of the compound tested is listed in

TABLE I. Chemical Structure of Disulfide Derivatives

General structure	Compound No.	R ₁	R ₂
I [6-MP]	-H	-SH
	II	-H	-SS-CH ₂ CH ₃
	III	-H	-SS-[CH ₂] ₂ CH ₃
	IV	-H	-SS-CH-CH ₃ CH ₃
	V	-H	-SS-[CH ₂] ₃ CH ₃
	VI	-H	-SS-[CH ₂] ₄ CH ₃
	VII	-H	-SS-[CH ₂] ₅ CH ₃
	VIII	-H	-SS-[CH ₂] ₆ CH ₃
	IX	-H	-SS-[CH ₂] ₇ CH ₃
	X	-H	-SS-[CH ₂] ₉ CH ₃
	XI	-H	-SS-[CH ₂] ₁₃ CH ₃
	XII	-H	-SS-CH ₂ -
	XIII	-H	-SS-CH ₂ -
	XIV [6-TG]	-NH ₂	-SH
	XV	-NH ₂	-SS-CH ₂ CH ₃
	XVI	-NH ₂	-SS-[CH ₂] ₂ CH ₃
	XVII	-NH ₂	-SS-CH-CH ₃ CH ₃
	XVIII	-NH ₂	-SS-[CH ₂] ₃ CH ₃
	XIX	-NH ₂	-SS-CHCH ₂ CH ₃ CH ₃
	XX	-NH ₂	-SS-CCH ₃ CH ₃
	XXI	-NH ₂	-SS-[CH ₂] ₄ CH ₃
	XXII	-NH ₂	-SS-[CH ₂] ₅ CH ₃
	XXIII	-NH ₂	-SS-[CH ₂] ₆ CH ₃
	XXIV	-NH ₂	-SS-[CH ₂] ₇ CH ₃
	XXV	-NH ₂	-SS-[CH ₂] ₉ CH ₃
	XXVI	-NH ₂	-SS-[CH ₂] ₁₃ CH ₃
	XXVII	-NH ₂	-SS-CH ₂ -

Table I. was assayed in ascites sarcoma-180 system as
Animal Experiments — Antitumor activity reported previously.⁷⁾ Six mice were used for each

TABLE II. *Antitumor Effect of Disulfide Derivatives of 6-MP and 6-TG in S-180A System*

Compound No.	Dose [mg/kg/day]	Tumor growth ^{a)} [T/C%]	Mortality ^{b)} [died/used]	Antitumor activity ^{c)}
I [6-MP]	400	-	6/6	Toxic
	320	18	5/6	Toxic
	300	1	1/6	Toxic
	250	7	0/6	+++
	180	1	0/6	+++
	100	19	0/6	++
	60	41	0/6	++
	30	63	0/6	+
	10	120	0/6	-
	30	-	6/6	Toxic
II	20	0	4/6	Toxic
	10	75	0/6	-
III	100	-	6/6	Toxic
	30	0	4/6	Toxic
	10	28	0/6	++
	3	60	0/6	+
IV	1	92	0/6	-
	50	0	5/6	Toxic
	30	1	1/6	Toxic
	10	16	0/6	++
V	3	84	0/6	-
	60	-	6/6	Toxic
	30	0	4/6	Toxic
	10	11	0/6	++
VI	3	95	0/6	-
	170	-	6/6	Toxic
	100	1	2/6	Toxic
	60	0	0/6	+++
VII	30	85	0/6	-
	100	-	6/6	Toxic
	60	3	0/6	+++
	30	4	0/6	+++
VIII	10	113	0/6	-
	200	0	4/6	Toxic
	170	0	3/6	Toxic
	100	2	0/6	+++
IX	30	21	0/6	++
	10	108	0/6	-
	200	1	5/6	Toxic
	100	4	0/6	+++
X	60	7	0/6	+++
	30	138	0/6	-
	150	0	5/6	Toxic
	120	2	0/6	+++
XI	100	1	0/6	+++
	60	0	0/6	+++
	30	52	0/6	+
	10	80	0/6	-
XII	300	1	3/6	Toxic
	150	55	0/6	+
	100	45	0/6	+
	60	58	0/6	+
XIII	30	86	0/6	-
	200	1	2/6	Toxic
	150	3	0/6	+++
	100	0	0/6	+++
XIII	60	0	0/6	+++
	30	22	0/6	++
	10	102	0/6	-
	150	8	0/6	+++
XIII	100	10	0/6	+++
	60	63	0/6	+
	30	54	0/6	+

Compound No.	Dose [mg/kg/day]	Tumor growth ^{a)} [T/C%]	Mortality ^{b)} [died/used]	Antitumor activity ^{c)}
XIV [6-TG]	60	-	6/6	Toxic
	30	1	0/6	+++
	10	1	0/6	+++
	3	52	0/6	+
	1	64	0/6	+
XV	30	10	3/6	Toxic
	10	23	0/6	++
	3	78	0/6	-
XVI	30	17	3/6	Toxic
	10	16	0/6	++
	3	43	0/6	+
XVII	1	84	0/6	-
	60	-	6/6	Toxic
	30	0	0/6	+++
	10	2	0/6	+++
	3	57	0/6	+
XVIII	1	68	0/6	-
	30	-	6/6	Toxic
	10	21	1/6	Toxic
XIX	3	76	0/6	-
	60	4	3/6	Toxic
	30	0	0/6	+++
	10	14	0/6	++
XX	3	45	0/6	+
	1	64	0/6	+
	100	0	4/6	Toxic
	60	0	0/6	+++
	30	18	0/6	++
XXI	10	25	0/6	++
	3	55	0/6	+
	1	85	0/6	-
	30	7	3/6	Toxic
XXII	10	29	0/6	++
	3	62	0/6	+
	60	0	4/6	Toxic
	30	14	3/6	Toxic
XXIII	10	13	0/6	++
	3	55	0/6	+
	60	0	5/6	Toxic
	30	17	4/6	Toxic
XXIV	10	21	0/6	++
	3	28	0/6	++
	1	82	0/6	-
	60	0	5/6	Toxic
XXV	30	9	1/6	Toxic
	10	1	0/6	+++
	3	29	0/6	++
	1	62	0/6	+
XXVI	50	0	4/6	Toxic
	30	24	2/6	Toxic
	10	16	0/6	++
	3	31	0/6	++
XXVII	1	61	0/6	+
	150	-	6/6	Toxic
	120	3	3/6	Toxic
	60	10	0/6	+++
XXVIII	30	54	0/6	+
	10	97	0/6	-
	90	0	5/6	Toxic
	60	0	0/6	+++
	30	9	0/6	+++
	10	19	0/6	++
	3	26	0/6	++
XXIX	1	88	0/6	-

a) Total packed cell volume in the treated mice is expressed as percentage of that in controls.

b) Ratio of dead animals to total animals used at the day of evaluation.

c) Antitumor activity was graded as: + + +, 10–0; ++, 40–11; +, 65–41; -, 100–66 of T/C%.

group. Ascites cells of 5×10^6 were inoculated intraperitoneally to female ICR/JCL mice of about 5 weeks old. Compounds to be tested were injected intraperitoneally in suspension with 0.5% sodium carboxymethyl cellulose in 0.85% saline. The injection was performed once daily for 5 days, starting 24 hr after tumor inoculation.

Evaluation of Antitumor Activity and Toxicity — Antitumor activity was evaluated by total packed cell volume (TPCV) ratio (T/C%) on the 7th day after tumor inoculation.⁷⁾ Antitumor activity was graded as +++10-0, ++40-11, +65-41, -100-66 of T/C%. ED₅₀ (dosage shown 50% inhibition in tumor growth) was calculated with log-probability plot of dosage-activity data. LD₅₀ of tumor bearing mice was calculated by the method of Weil⁸⁾ with the 7th day survivors. Chemotherapeutic index (LD₅₀/ED₅₀) was also calculated.

RESULTS

Antitumor Activity of the Disulfide Derivatives of 6-Mercaptopurine

As shown in Table II (I–XIII), almost all the derivatives were moderately (++) or markedly (+++) active against ascites form of sarcoma-180 at optimal dosage. Effect of α -naphthyl derivative (XII) was the highest and tumor growth was completely inhibited at non-lethal dosage. ED₅₀ and LD₅₀ of the derivatives on molar basis are shown in Fig. 1. Most derivatives were more active than the parent compound, 6-MP. Activity of the compounds reached maximum with propyl derivative (III) and declined gradually along with the extension of side chain. Activity of the most active propyl derivative (III) was 15 times that of 6-MP.

Toxicity of the compound was also changed along with the extension of side chain in a similar pattern. As a result, only α -naphthyl derivative (XII) showed a slightly higher chemotherapeutic index of 8.7 than that of parent compound (7.5) (Fig. 2, a).

Antitumor Activity of the Disulfide Derivatives of 6-Thioguanine

As shown in Table II (XIV–XXVII), all the

FIG. 1. Chemical Structure-Antitumor Activity [ED₅₀] Relationship and Chemical Structure-Toxicity [LD₅₀] Relationship in Disulfide Derivatives of 6-MP

Compounds were injected intraperitoneally once daily for 5 days, starting 24 hr after tumor inoculation

○ □; ED₅₀ [μmol/kg/day], ● ■; LD₅₀ [μmol/kg/day] □; iso-C₃H₇, Bz; benzyl, Na; naphthyl.

derivatives except butyl derivative (XVIII) were active at optimal dosage. Effect of iso-propyl (XVII), *sec*-butyl (XIX), *tert*-butyl (XX), and α -naphthyl (XXVII) derivatives was marked and tumor growth was completely inhibited at non-lethal dosage.

In quantitative analysis of activity and toxicity on molar basis, it was revealed that activity of the compounds was increased along with the extension of side chain to 10 in carbon number in linear alkyl derivatives but tetradecyl derivative (XXVI) was less active than 6-TG (Fig. 3). Activity of the most active decyl derivative (XXV) was 4 times that of 6-TG. Toxicity of the compounds was increased by modification of chemical structure and it was less than 4 times that of 6-TG. Decyl (XXV) and α -naphthyl (XXVII) derivatives were similarly toxic to mice as the parent

FIG. 2. Relationship between Chemical Structure and Chemotherapeutic Index $[LD_{50}/ED_{50}]$ in Disulfide Derivatives of 6-MP [a] and 6-TG [b]

○; linear side chain, □; iso-C₃H₇, △; sec-C₄H₉, ▽ tert-C₄H₉, Bz; benzyl, Na; Naphthyl.

FIG. 3. Chemical Structure-Antitumor Activity $[ED_{50}]$ Relationship and Chemical Structure-Toxicity $[LD_{50}]$ Relationship in Disulfide Derivatives of 6-TG

Injection procedure was same as described in Fig. 1.

○ □ △ ▽; ED_{50} [μ mol/kg/day], ● ■ ▲ ▼; LD_{50} [μ mol/kg/day] □; iso-C₃H₇, △; sec-C₄H₉, ▽; tert-C₄H₉, Bz; benzyl, Na; naphthyl.

compound.

As a result, chemotherapeutic indices of the majority of the derivatives were smaller than that of 6-TG (Fig. 2, b). However, the indices of sec-butyl (XIX), tert-butyl (XX), octyl (XXIV), decyl (XXV), and α -naphthyl (XXVII) derivatives were 27, 24, 23, 26, and 28, respectively. They were higher than that of 6-TG (14).

DISCUSSION

To improve the therapeutic effect of mercapto purines, several modifications have been examined and Imuran is successfully applied for clinical use as an immunosuppressor in organ transplantation but not as an antileukemic agent.⁹ In the present experiments, chemotherapeutic index was increased by the modification of mercapto to alkyl disulfide in 6-TG but not in 6-MP. The improvement of 6-TG was made in terms of increase in activity but not decrease in toxicity unlike in the case of alkylthio modification where toxicity to host was decreased.¹⁰ The difference is perhaps explained by the fact that disulfide bond is more labile than alkylthio bond in the presence of reducing agents

and the parent compound tends to be released rapidly. Octyl (XXIV), decyl (XXV), *sec*-butyl (XIX), *tert*-butyl (XX), and α -naphthyl (XXVII) derivatives of 6-TG showed higher chemotherapeutic indices than 6-TG. It suggests that lypophyllicity of side chain is valuable to improve the therapeutic effect in 6-TG. These five derivatives are selected as the best candidates for further evaluation.

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