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Mutagenicity of Several Classes of Antitumor Agents to *Salmonella typhimurium* TA98, TA100, and TA92[1]

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ABSTRACT

The mutagenic activities of antitumor agents, including 5 antibiotics, 19 antimetabolites, 5 alkylating agents, 2 alkaloids, 1 enzyme, and 1 adrenal steroid hormone, were tested on *Salmonella typhimurium* TA100, TA98, and TA92.

Four of these, busulfan, carbazilquinone, 1-(4-amino-2-methylpyrimidine-5-yl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride, and pipobroman were shown for the first time to be mutagenic. Further, the known mutagenicities of five others, daunomycin hydrochloride, Adriamycin hydrochloride, mitomycin C, 6-mercaptopurine, and cyclophosphamide, were confirmed.

INTRODUCTION

Many chemotherapeutic agents are now widely used clinically for treatment of cancers and leukemias and sometimes for prophylaxis of recurrent cancer after surgical treatment.

However, a phenomenon known as Haddow's paradox (5) is that some cancer chemotherapeutic agents are carcinogenic to laboratory animals and also induce chromosomal aberrations in cultured mammalian cells. Thus, it is important to know whether chemotherapeutic agents are carcinogenic.

Recently, it has become widely accepted that many mutagens are carcinogens (15, 16, 24). Therefore, in this work we tested the mutagenic activities in *S. typhimurium* strains TA100, TA98, and TA92 of various kinds of chemotherapeutic agents now in use or being tested for use in treatment of human cancer. The compounds tested were antibiotics, antimetabolites, alkylating agents, alkaloids, an enzyme, and a hormone.

MATERIALS AND METHODS

Microbes. *S. typhimurium* strains TA100, TA98, and TA92 were kindly supplied by Dr. Bruce N. Ames, University of California, Berkeley, Calif. TA100 and TA98 are *uvrB* and *rfa* (deep rough) mutants (1). TA92 has the capacity for excision repair and an intact lipopolysaccharide barrier (LPS^+) (1). TA100 and TA92 both contain the same base-pair change mutation, and TA98 contains a frameshift mutation at *his* locus (1). All these strains carry R-factor plasmid pKM101 (1).

Chemicals. Adriamycin hydrochloride, 5-fluorouracil, mitomycin C, and L-asparaginase were obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Cyclophosphamide and vinblastine sulfate were purchased from Shionogi & Co., Ltd., Osaka, Japan; Methotrexate and busulfan were from Takeda Chemical Industries, Ltd., Osaka, Japan; 1-β-D-arabinofuranosylcytosine and cyclocytidine were from Kohjin Co., Ltd., Tokyo, Japan. 6-Mercaptopurine and 8-azaguanine were from Sigma Chemical Co., St. Louis, Mo.; ACNU[3] and carbazilquinone were from Sankyo Co., Ltd., Tokyo, Japan; and actinomycin D was from Nippon Merck-Banyu Co., Ltd., Tokyo, Japan. Bleomycin hydrochloride was from Nippon Kayaku Co., Ltd., Tokyo, Japan; daunomycin hydrochloride was from Meiji Seika Kaisha, Tokyo, Japan; and FT-207 was from Taiho Pharmaceutical Co., Ltd., Tokyo, Japan. Alkylcarbamoyl derivatives of 5-fluorouracil were synthesized at Mitsui Pharmaceuticals, Inc., Tokyo, Japan. Vincristine sulfate was from Eli Lilly & Co., Indianapolis, Ind., and pipobroman was from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. Prednisolone was from Sanwa Research Institute, Tokyo, Japan. Glucose 6-phosphate, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and ATP were from Sigma; NADH and NADPH were from Oriental Yeast Co., Ltd., Tokyo, Japan; and dimethyl sulfoxide, spectrophotometric grade, was from Wako Pure Chemical, Inc., Osaka, Japan.

Eight compounds for drug use contained some vehicle, and the amounts of these compounds were expressed as μg of active principle. The names of these compounds and the amounts in 1 vial, 1 ampul, or 1 tablet were as follows: daunomycin·HCl (20 mg principle; actual weight, 120 mg/vial), Adriamycin·HCl (10 mg principle; actual weight, 50 mg/vial), bleomycin·HCl (15 mg potency; actual weight, 8 mg/ampul), actinomycin D (0.5 mg principle; actual weight, 20.5 mg/vial), mitomycin C (0.996 mg principle; actual weight, 1 mg/vial), cyclophosphamide (500 mg principle; actual weight, 725 mg/vial), L-asparaginase (10,000 IU; actual weight, 33 mg/vial), and prednisolone (5 mg principle; actual weight, 153 mg/tablet).

Mutation Test. The mutation test was carried out by our modification (20) of the method of Ames *et al.* (1). The test chemical in 0.1 ml dimethyl sulfoxide or H_2O was placed in a tube and mixed with 0.5 ml S-9 mix (150 μl of the S-9 fraction of rat liver pretreated with polychlorinated biphenyl, 2 μmol NADPH, 2 μmol NADH, 2.5 μmol glucose 6-phosphate, 0.25 unit glucose-6-phosphate dehydrogenase, 4 μmol $MgCl_2$, 16.5 μmol KCl, and 50 μmol sodium phosphate buffer, pH 7.4 and 0.1 ml of culture of the bacterial

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[3] The abbreviations used are: ACNU, 1-(4-amino-2-methylpyrimidine-5-yl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride; FT-207, 1-(2-tetrahydrofuryl)-5-fluorouracil.

tester strain (1 to 2 × 10^8 cells). Without metabolic activation 50 μmol sodium phosphate buffer, pH 7.4, in 0.5 ml were used instead of S-9 mix. The mixture was preincubated at 37° for 20 min and then mixed with 2 ml top agar (0.7% agar and 0.6% NaCl) at 45° and spread on a minimal-glucose agar plate containing 0.1 μmol each L-histidine and biotin. Plates were incubated at 37° for 2 days, and then *his*$^+$ revertant colonies were counted.

RESULTS AND DISCUSSION

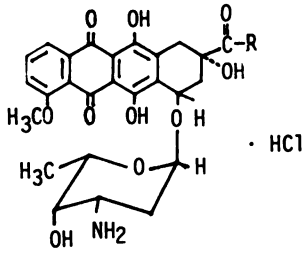
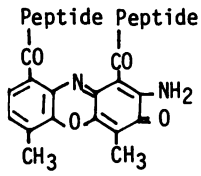
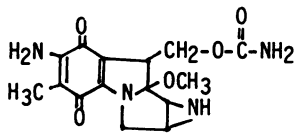
The trivial names and structures of the 5 antibiotics, 19 antimetabolites, 5 alkylating agents, and other compounds tested are shown in Tables 1, 3, 5, and 7, respectively, with the results of mutagenicity tests on them and information on their carcinogenicities.

Antibiotics. Results on the mutagenicities of the antibiotics are listed in Table 2. The mutagenicities of daunomycin hydrochloride (16), Adriamycin hydrochloride (16), and mitomycin C (17), which had been reported previously, were confirmed. Daunomycin hydrochloride and Adriamycin hydrochloride intercalate DNA (33), resulting in inhibition of DNA-dependent DNA and RNA polymerases (32). These compounds were highly mutagenic to TA100 and TA98 without S-9 mix, the latter showing more mutagenicity in TA98 than in TA100. Addition of S-9 mix reduced the activity. Clear dose-response activities of these compounds were obtained, as shown in Table 2. These compounds have potent actions in producing malignant transformation *in vivo* and *in vitro* and mutation in mammalian cells *in vitro* (14). Bleomycin hydrochloride was not mutagenic to TA100 or TA98, with or without S-9 mix, but it is known to cause DNA strand breakage (26). Actinomycin D, which binds preferentially to dG-dC pairs of DNA (13) and inhibits DNA-dependent RNA synthesis (12), was not mutagenic to TA100 or TA98 with or without S-9 mix. The *in vivo* carcinogenicity of actinomycin D in rats has been reported (30). Actinomycin D is also reported to have very weak mutagenic activity on mammalian cells cultured *in vitro* (14). Mitomycin C was not mutagenic to TA100 or TA98 but was weakly mutagenic to TA92, especially without S-9 mix, as shown in Table 2. Mitomycin C produces interstrand cross-linking of double-stranded DNA (27). In this case *uv* repair-deficient strain cells were killed and did not yield any mutants (19). TA92, which has *uv* repair capacity, gave more revertants than did the spontaneous revertants. Mitomycin C produces tumors *in vivo* (30). Bleomycin hydrochloride and actinomycin D were not mutagenic but were toxic to TA100 and TA98, as was mitomycin C. But these compounds were not mutagenic to TA92 (Table 2).

Antimetabolites. Results on antimetabolites are listed in Table 4. 5-Fluorouracil, an inhibitor of thymidylate synthetase, was not mutagenic to TA100 or TA98 with or without S-9 mix, as shown in Table 4. 5-Fluorouracil had a strong lethal effect on cells at a concentration of 5 μg/plate without S-9 mix. FT-207 also was not mutagenic to TA100 or TA98 with or without S-9 mix. These 2 compounds were also not mutagenic to TA92 with or without S-9 mix.

Table 1

Trivial names, structures, mutagenicities, and carcinogenicities of antibiotics

Trivial name	Structure	Mutagenicity	Carcinogenicity
Daunomycin·HCl		+	+ (14)
Adriamycin·HCl	*R*: —CH_3, daunomycin —CH_2OH, Adriamycin	+	+ (14)
Bleomycin·HCl	Glycopeptide	–	
Actinomycin D		–	+ (30)
Mitomycin C[a]		+	+ (30)

[a] Mitomycin C is mutagenic to *S. typhimurium* TA92.

Table 2
Results of mutation tests on antibiotics

Drug[a]	μg/plate	*his*⁺ revertants/plate TA100 + S-9 mix	TA100 − S-9 mix	TA98 + S-9 mix	TA98 − S-9 mix	TA92 + S-9 mix	TA92 − S-9 mix	*his*⁺ revertants/nmol[b]
Daunomycin·HCl	0.083	144	286	24	340			
	0.17	160	295	27	852			2800 (8, −)[c]
	0.42	163	202	33	1555			
	0.83	147	144	62	1368			
Adriamycin·HCl	0.02	109	106	24	102			
	0.05	130	309	28	323			
	0.1	152	234	27	556			
	0.2	159	98	22	1034			
	0.5	270	72	31	776			3130 (8, −)
	1	303	36	55	260			
	2	170	20	106	64			
	5	185	67	423	45			
	10	168	0	443	0			
Bleomycin·HCl	0.94	99	190	25	17	46	39	
	1.88	111	143	23	18	48	37	
	4.7	125	10	20	7	49	32	0
	9.4	129	0	22	0	49	28	
	18.8	106	0	12	0	45	9	
Actinomycin D	2.4	125	103	18	18	28	24	
	6.1	100	90	17	15	20	34	0
	12.2	66	45	21	18	14	32	
Control[d]		147	132	30	16			
Mitomycin C	0.01	147	115	21	26	48	36	
	1.0	110	0	21	0	48	90	
	2.5	70	0	21	0	40	120	43 (2, −)
	5	75	0	22	0	80	137	
	10	103	0	17	0	61	72	
Control[e]		131	122	19	17	39	26	

[a] Drug contained some vehicle, and the amount of the drug was expressed as μg of active principle.
[b] The number of *his*⁺ revertants per nmol was calculated from the data obtained under the most active conditions in the linear dose-responsible range and a yield of 100 colonies more than the background yield.
[c] Tester strain TA98; −, without S-9 mix; 2, TA92.
[d] Average of values in 20 experiments. The numbers of revertants were: 117 to 183 of TA100 with S-9 mix, 89 to 169 of TA100 without S-9 mix, 19 to 38 of TA98 with S-9 mix, and 14 to 26 of TA98 without S-9 mix.
[e] Average of values in 2 experiments.

Twelve alkylcarbamoyl derivatives of 5-fluorouracil (namely, 1-methyl, 1-ethyl, 1-propyl, 1-isopropyl, 1-butyl, 1-*tert*-butyl, 1-pentyl, 1-hexyl, 1-cyclohexyl, 1-phenyl, 1-heptyl, and 1-octyl-carbamoyl-5-fluorouracil) were all nonmutagenic to TA100 and TA98 with or without S-9 mix. All of these derivatives were cytotoxic at doses of 2.5 to 5 μg/plate. Therefore, these compounds were also tested in TA92 and were found not to be mutagenic, as shown in Table 4. These alkylcarbamoyl derivatives of 5-fluorouracil are not yet used clinically, although their antitumor activities against experimental tumors in mice were demonstrated by Hoshi *et al.* (9, 10).

6-Mercaptopurine, an inhibitor of purine synthesis, was weakly mutagenic to TA100 without S-9 mix, as shown in Table 4. This drug was reported to be mutagenic to *S. typhimurium* strains TA1535 and *his*G46 by the liquid method (8). The noncarcinogenicity of this compound was reported (25). Methotrexate, which inhibits the biosyntheses of purine and thymine from formate, gave negative results on TA100 and TA98 at doses of up to 312 μg/plate. It had a slight lethal effect on the cells. Methotrexate was also reported to be nonmutagenic to *S. typhimurium* TA1535, TA1536, TA1537, TA1538, and *his*G46 by the liquid method (8). A weak carcinogenicity of this compound on experimental animals has been reported (22), but it has also been reported not to be carcinogenic (23) and to cause no chromosomal aberrations in mammalian culture cells (7). Thus, further studies are required to determine whether it is really carcinogenic.

8-Azaguanine, an inhibitor of guanylic acid synthesis, was not mutagenic to TA100, TA98, or TA92 with or without S-9 mix (Table 4). It had no effect on cell survival of TA100 or TA92 at doses of up to 50 μg/plate but killed TA98 at a dose of 25 μg/plate. This compound is noncarcinogenic (6).

1-β-D-Arabinofuranosylcytosine and cyclocytidine were not mutagenic at doses of up to 30 and 25 mg/plate, respectively (Table 4). Arabinofuranosylcytosine is known to interfere with DNA polymerase (18) and to be incorporated into DNA (31), and it induced chromosomal aberrations both *in vivo* (2) and *in vitro* (4, 11).

Alkylating Agents. Five alkylating agents were all found to be mutagenic in this system, as shown in Table 6. Cyclophosphamide was mutagenic to TA100 with S-9 mix but was not mutagenic to TA98. This compound was reported to be mutagenic (8, 16) and carcinogenic (29). Busulfan was mutagenic to TA100 with or without S-9 mix but not to TA98. Busulfan was reported to be carcinogenic

Table 3

Trivial names, structures, mutagenicities, and carcinogenicities of antimetabolites

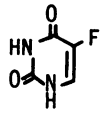
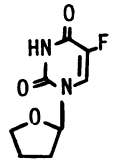
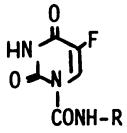
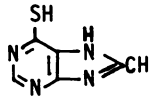
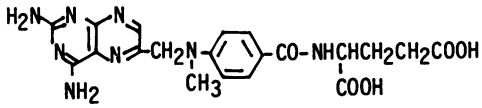
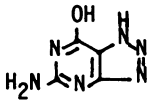
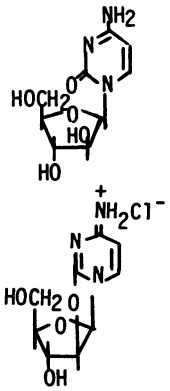
Trivial name	Structure	Mutagenicity	Carcinogenicity
5-Fluorouracil		–	
FT-207		–	
Carbamoyl derivatives of 5-fluorouracil			
R: Methyl		–	
Ethyl		–	
Propyl		–	
Isopropyl		–	
Butyl		–	
tert-Butyl		–	
Pentyl		–	
Hexyl		–	
Cyclohexyl		–	
Phenyl		–	
Heptyl		–	
Octyl		–	
6-Mercaptopurine		+	– (25)
Methotrexate		–	+ (22), – (23)
8-Azaguanine		–	– (6)
1-β-D-Arabinofuranosylcytosine		–	
Cyclocytidine		–	

Table 4
Results of mutation tests on antimetabolites

Antimetabolite	μg/plate	*his*⁺ revertants/plate						*his*⁺ revertants/nmol[a]
		TA100		TA98		TA92		
		+ S-9 mix	− S-9 mix	+ S-9 mix	− S-9 mix	+ S-9 mix	− S-9 mix	
5-FU[b]	1	183	82	30	13	33	19	
	5	134	0	32	0	32	0	0
	10	121	0	38	0	0	0	
	50	133	0	0	0	0	0	
FT-207	25	132	99	37	7	34	15	
	50	131	101	14	1	21	13	0
	250	87	0	0	0	22	9	
1-Methylcarbamoyl-5-FU	1	177	125	35	11	19	19	
	5	153	0	49	0	21	0	0
	10	173	0	27	0	17	0	
	50	0	0	0	0	6	0	
1-Ethylcarbamoyl-5-FU	2.5	145	125	27	14	26	16	
	5	165	0	27	7	16	0	0
	10	142	0	22	0	15	0	
	50	100	0	0	0	1	0	
1-Propylcarbamoyl-5-FU	2.5	158	133	28	10	20	12	
	5	189	0	32	0	20	0	0
	10	137	0	31	0	11	0	
	50	0	0	0	0	1	0	
1-Isopropylcarbamoyl-5-FU	0.5	129	103	14	9	26	18	
	2.5	134	0	25	0	23	0	0
	10	194	0	20	0	18	0	
	50	97	0	40	0	1	0	
1-Butylcarbamoyl-5-FU	0.5	162	154	24	21	26	17	
	2.5	170	96	23	0	30	0	0
	5	150	0	29	0	17	0	
	50	140	0	22	0	2	0	
1-*tert*-Butylcarbamoyl-5-FU	0.5	110	100	12	12	27	10	
	2.5	126	0	21	0	28	0	0
	10	142	0	24	0	30	0	
	50	160	0	0	0	3	0	
1-Pentylcarbamoyl-5-FU	0.5	170	118	36	21	21	13	
	2.5	162	125	19	0	29	0	0
	5	171	0	30	0	22	0	
	50	154	0	21	0	13	0	
1-Hexylcarbamoyl-5-FU	0.5	120	71	26	10	30	19	
	2.5	107	0	29	0	22	0	0
	5	114	0	16	0	27	0	
	10	148	0	24	0	3	0	
1-Cyclohexylcarbamoyl-5-FU	0.5	132	104	30	15	24	13	
	1	149	115	37	11	25	0	0
	5	146	0	35	0	23	0	
	10	138	0	31	0	2	0	
1-Phenylcarbamoyl-5-FU	0.5	121	110	27	9	25	13	
	2.5	151	0	17	0	13	0	0
	10	131	0	22	0	30	0	
	50	67	0	0	0	2	0	
1-Heptylcarbamoyl-5-FU	0.5	174	95	33	12	25	18	
	2.5	146	0	19	0	17	0	0
	10	151	0	29	0	17	0	
	50	0	0	0	0	12	0	

Table 4—*Continued*

Antimetabolite	μg/plate	*his*⁺ revertants/plate: TA100 + S-9 mix	TA100 − S-9 mix	TA98 + S-9 mix	TA98 − S-9 mix	TA92 + S-9 mix	TA92 − S-9 mix	*his*⁺ revertants/nmol[a]
1-Octylcarbamoyl-5-FU	0.5	130	80	28	8	33	15	
	2.5	135	0	22	0	31	0	0
	10	130	0	31	0	13	0	
	50	0	0	0	0	11	0	
6-Mercaptopurine	250	114	276	22	20			
	500	110	313	32	21			0.06[c]
	750	127	282	24	9			
	1,000	90	231	24	6			
Methotrexate[d]	104	142	65	36	33			
	208	172	77	35	35			0
	312	169	85	37	21			
8-Azaguanine	10	109	117	44	20	21	14	
	25	126	139	37	0	36	19	0
	50	123	127	36	0	33	18	
1-β-D-Arabinofuranosylcytosine	1,000	127	92	37	31			
	5,000	145	134	30	30			
	10,000	158	125	28	22			0
	25,000	135	132	34	19			
	30,000	131	141	28	20			
Cyclocytidine	5,000	127	90	29	22			
	10,000	91	77	19	25			0
	20,000	132	105	31	37			
	25,000	150	135	38	22			
Control[e]		147	132	30	16	39	26	

[a] The number of *his*⁺ revertants per nmol was calculated from the data obtained under the most active conditions in the linear dose-responsible range and a yield of 100 colonies more than the background yield.
[b] 5-FU, 5-fluorouracil.
[c] Tester strain TA100 without S-9 mix.
[d] Drug contained some vehicle, and the amount of the drug was expressed as μg of active principle.
[e] Average of values in 20 experiments. The numbers of revertants were: 117 to 183 of TA100 with S-9 mix, 89 to 169 of TA100 without S-9 mix, 19 to 38 of TA98 with S-9 mix, and 14 to 26 of TA98 without S-9 mix.

(28). ACNU, which is now being tested clinically, was mutagenic to TA100 with or without S-9 mix but was not mutagenic to TA98 at doses of up to 50 μg/plate. Carbazilquinone was mutagenic to TA100 with or without S-9 mix. Pipobroman was mutagenic to TA100 without S-9 mix but was not mutagenic to TA98.

***Vinca* Alkaloids.** Results on *Vinca* alkaloids, including vinblastine sulfate and vincristine sulfate, are shown in Table 8. These compounds were not mutagenic to TA100 or TA98 with or without S-9 mix at doses of up to 500 μg/plate, as shown in Table 8. Moreover, a dose of 500 μg/plate had no apparent lethal effect on the bacterial cells. These alkaloids were reported to inhibit the growth of cells in culture (21), but no data on their carcinogenicities are available.

Enzyme. L-Asparaginase was not mutagenic to either strain with or without S-9 mix (Table 8). Moreover, it had no lethal effect at doses of up to 3 mg/plate.

Adrenal Steroid Hormone. Prednisolone was not mutagenic to either strain with or without S-9 mix (Table 8), and it was not lethal at doses of up to 33 μg/plate. This compound was not tested at concentrations greater than 33 μg/plate.

In this work we tested the mutagenicities of various kinds of antitumor agents. Some of them are now in clinical use, and some, such as various alkylcarbamoyl-5-fluorouracil derivatives, are now being tested in animals. Among 33 compounds tested 9 were found to be mutagenic; these were daunomycin hydrochloride, Adriamycin hydrochloride, mitomycin C, 6-mercaptopurine, cyclophosphamide, busulfan, ACNU, carbazilquinone, and pipobroman. The mutagenicities of daunomycin hydrochloride (3, 16), Adriamycin hydrochloride (3, 16), 6-mercaptopurine (3, 8), and cyclophosphamide (3, 8, 16) were reported, and the lack of mutagenicity found with methotrexate (3, 8) and actinomycin D (3) were confirmed in this study. Recently, Benedict *et al.* (3) reported that uracil mustard was mutagenic to TA1535 without S-9 mix. Uracil mustard has a bis(2-chloroethyl)amino group. This bis(2-chloroethyl)amino group may alkylate DNA and induce mutation. On the other hand, 5-fluorouracil and its alkylcarbamoyl derivatives seem to have no alkylating activity.

Certain substances used in cancer chemotherapy are endowed with mutagenic potential. There is a demonstrated correlation between mutagenicity in this bacterial test system and potential for causing cancer in laboratory animals. Therefore, the development of new chemotherapeutic agents that have no mutagenicity in such bacterial

Table 5

Trivial names, structures, mutagenicities, and carcinogenicities of alkylating agents

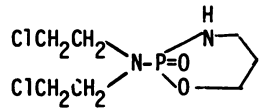
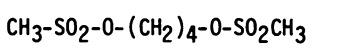
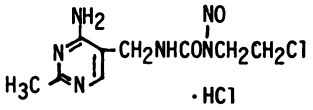
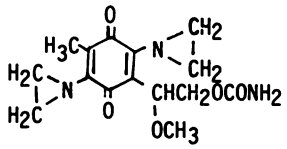
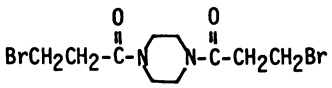
Trivial name	Structure	Mutagenicity	Carcinogenicity
Cyclophosphamide		+	+ (29)
Busulfan		+	+ (28)
ACNU		+	
Carbazilquinone		+	
Pipobroman		+	

Table 6

Results of mutation tests on alkylating agents

Agent	μg/plate	*his*⁺ revertants/plate: TA100 + S-9 mix	TA100 − S-9 mix	TA98 + S-9 mix	TA98 − S-9 mix	*his*⁺ revertants/nmol[a]
Cyclophosphamide[b]	70	158	90	16	13	
	175	309	110	27	5	0.26 (0, +)[c]
	525	437	106	22	2	
	700	401	133	25	0	
Busulfan	100	160	137	35	3	
	500	390	267	24	4	0.14 (0, +)
	750	578	578	35	7	
	1000	1132	1038	49	5	
ACNU	5	140	182	30	21	
	10	178	218	28	23	2.33 (0, −)
	25	234	262	23	23	
	50	500	366	38	30	
Carbazilquinone	10	198	365	29	26	
	25	251	602	40	48	7.48 (0, −)
	75	273	641	66	89	
	100	317	420	29	85	
Pipobroman	250	127	176	35	31	
	500	147	218	35	29	0.061 (0, −)
	750	157	260	30	26	
	1000	196	313	38	24	
Control[d]		147	132	30	16	

[a] The number of *his*⁺ revertants per nmol was calculated from the data obtained under the most active conditions in the linear dose-responsible range and a yield of 100 colonies more than the background yield.

[b] Drug contained some vehicle, and the amount of drug was expressed as μg of active principle.

[c] 0, Tester strain TA100; +, with S-9 mix; −, without S-9 mix.

[d] Average of values in 20 experiments. The numbers of revertants were: 117 to 183 of TA100 with S-9 mix, 89 to 169 of TA100 without S-9 mix, 19 to 38 of TA98 with S-9 mix, and 14 to 26 of TA98 without S-9 mix.

Table 7

Trivial names, structures, and mutagenicities of Vinca alkaloids, an enzyme, and an adrenal steroid hormone

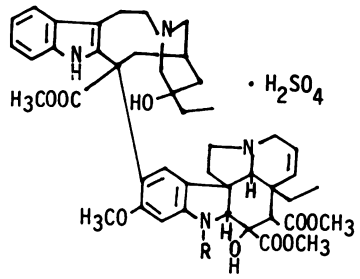
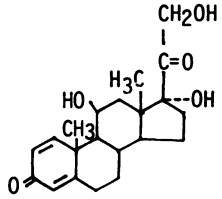
Trivial name	Structure	Mutagenicity
Vinblastine sulfate		–
Vincristine sulfate		–
	R: $-CH_3$ Vinblastine $-CHO$ Vincristine	
L-Asparaginase		–
Prednisolone		–

Table 8

Results of mutation tests on Vinca alkaloids, an enzyme, and an adrenal steroid hormone

		his⁺ revertants/plate				
		TA100		TA98		
	μg/plate	+ S-9 mix	− S-9 mix	+ S-9 mix	− S-9 mix	*his*⁺ revertants/nmol[a]
Vinblastine sulfate	100	124	102	25	9	
	250	93	129	29	14	0
	500	92	113	26	14	
Vincristine sulfate	100	113	121	32	18	
	250	104	127	33	18	0
	500	149	126	26	16	
L-Asparaginase[b]	500 (152 IU)	146	134	29	15	
	1500 (455 IU)	148	156	23	26	0
	3000 (909 IU)	165	135	25	23	
Prednisolone[b]	8.3	135	125	37	34	
	17	145	145	39	33	0
	33	123	144	35	23	
Control[c]		147	132	30	16	

[a] The number of *his*⁺ revertants per nmol was calculated from the data obtained under the most active conditions in the linear dose-responsible range and a yield of 100 colonies more than the background yield.
[b] Drug contained some vehicle, and the amount of the drug was expressed as μg of active principle.
[c] Average of values in 20 experiments.

test systems would be most desirable because of the possibly decreased risk of latent drug-induced cancer in the patient undergoing chemotherapy.

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