Evaluation of the Genotoxic Potential of Newly Synthesized Triazoles and Hydrazides

Avaliação do Potencial Genotóxico de Quatro Quimioterápicos Inéditos Sintetizados a Partir de Triazóis e Hidrazidas

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Abstract

The mutagenic activity of four newly synthesized chemoterapic compounds, two from s-triazole (antiparasite actions) and two from hydrazides (tuberculostatic effects), was analyzed by the micronucleus assay in bone marrow of mice and by the Salmonella/microsome assay. In the micronucleus assay, the animals were dosed orally by gavage with maximum tolerated dose (MTD), single dose, and killed 24 and 48 hours after treatment. The Salmonella/microsome assay was carried out with a preincubation method and Salmonella typhimurium *strains TA98 and TA100. The results from the present study showed that both s-triazoles and both hydrazides did not induce genotoxicity. Consequently, these results led to continue the efforts to evaluate the pharmacological potentials of these products.*

Key words: *Ames test, genotoxicity, hydrazides, micronuclei, mutagenicity, triazoles*

Resumo

Neste trabalho foram avaliadas as atividades mutagênicas de quatro quimioterápicos novos, dois sintetizados a partir do triazol (ação antiparasitária) e dois das hidrazidas (efeito sobre a tuberculose). A avaliação mutagênica foi realisada utilizando-se o teste de micronúcleos em camundongos e também o ensaio Salmonella / Teste de Ames. Para o teste de micronúcleos utilizou-se dosagem oral da dose máxima tolerada, dosagem única (teste agudo), e sacrifício 24 e 48h após tratamento. Para o Teste de Ames utilizou-se duas linhagens de Salmonella

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typhimurium*, TA98 e TA100. Os resultados do presente estudo para ambos compostos testados, s-triazóis e hidrazidas, não mostram diferenças significativas em relação ao controle negativo, sendo assim estas drogas consideradas não-mutagênicas pelos presentes testes. Consequentemente, estes resultados permitem que sejam continuados os estudos dos potenciais farmacológicos destes produtos.*

Palavras-Chave: *Teste de Ames, genotoxicidade, hidrazidas, micronúcleo, mutagenicidade, triazóis*

Introduction

The use of genotoxic evaluation by the modern Pharmaceutic Industry increased very fast in the last decade. This was due to the necessity of toxicological evaluation of drugs and the search for less toxic compounds. The main interest of pharmaceutic activities is focussed on chemotherapic drugs. The triazoles, with their known antiparasite actions, and hydrazides, with tuberculostatic effects, are some of these drugs (YALE et al., 1953; PIGNATELO et al., 1991; BAJI et al., 1995; MIYAUCHI et al., 1995). Many of these compounds and their derivatives demonstrate structure-activity relationships in mutagenicity and carcinogenicity (CRAIG & ATITZEL, 1986; VELÁZQUEZ et al., 1990; BURLINSN et al., 1991; KNASMÜLLER & SZAKMARY, 1992; ALBANO et al., 1993; BARALE et al., 1993; ZANI et al, 1993; SILVA et al., 1994).

Enclosed in this general interest to search for less harmful, but highly active chemoterapic drugs, we analyzed the genotoxicity of the following new synthesized drugs: (1) 3-(3'-nitrophenyl)-1,2,4 triazole-[4,3-b]-benzothiazol (VN-3) and (2) 3-(4'-cyanophenyl)-1,2,4-triazole[4,3-b]benzothiazol (ECB-93), both derived from benzothiazol (triazole); and (3) 4-(3', 4'-methylenedioxybenzilidene)-pyridine carboxilic acid hydrazide (ECM-1) and (4) 4-(4'-nitrobenzilidene)-pyridine carboxilic acid hydrazide (ECM-4), both from isoniazid (hydrazide).

The mutagenic effect of these derivatives was analyzed by the micronucleus (MN) assay in mice, and by the Salmonella/microsome assay. The first detects clastogenic and aneugenic events, and the second gene mutations.

Material And Methods

Chemicals

The compounds 3-(3'-nitrophenyl)-1,2,4 triazole-[4,3-b]-benzothiazol and 3-(4'-cyanophenyl)-1,2,4-triazole[4,3-b]benzothiazol that were synthesized from 2-aminobenzothiazol, while 4-(3', 4'-methylenedioxybenzilidene)-pyridine carboxilic acid hydrazide and 4-(4'-nitrobenzilidene)-pyridine carboxilic acid hydrazide from isoniazide (Fig. 1). These compounds were synthesized by the Laboratory of Organic Drug Synthesis, Pharmacy Faculty of the Federal University of the State of Rio Grande do Sul.

Micronucleus Assay

Animals

Two mice strains were chosen: CF-1, for s-triazoles, and Swiss Webster, for hydrazides. CF-1 mice were supplied by the animal house of the Federal University of Rio Grande do Sul - UFRGS, and Swiss Webster by the Department of Agriculture. The animals were housed in propylene cages with wood shaving on the floor and caged by gender. Bottled tap water and rats diet (Nuvital, PR) were provided *ad libitum*. The room was maintained at stable temperature (23 to 25 ^{0}C). The mice were used in the test after, at least, a week of acclimation. The animals used weighted about 20 g, and were 5-7 weeks old. In the

Micronucleus Test, ten mice (five for each gender) per group of analysis were dosed.

In vivo mouse erythrocyte micronucleus assay

Each complete test was made according a report of the U.S. Environmental Protection Agency Gene-Tox program (MAVOURNIN et al., 1990). At first, preliminary tests were performed to evaluate the toxicity of the products. To determine the maximum tolerated dose (MTD), four mice were dosed with a single dose of 500, 1000 and 2000 mg/kg body weight, observed during a period of 72 h, for each drug. The MTD was defined by LD_{50} (letal dose) and observation of toxic effects. They were treated by oral gavage with a volume of 0,1 ml/10g of body weigh of mouse. In the test, the animals were dosed with determined MTD, and were killed 24 and 48 h after treatment. Besides the two test groups, negative (orally dosed with vehicle: for triazoles corn oil and for hidrazydes water) and positive (orally dosed with 60 mg/kg of cyclophosphamide (EDUXAN) diluted in water) controls were used. The bone marrow was extracted from the two femurs. Smear was made from material directly homogenized on two slides, with a drop of fetal calf serum. The slides were stained with 5% Giemsa, and coded for a "blind" analyzis. To avoid false negatives, the polychromatic erythrocyte (PCE): normochromatic erythrocyte (NCE) ratio in 1000 erythrocytes was determined, as a measure of toxicity of the product on the bone marrow. The frequency of micronucleated cells was analyzed in 2000 PCE/mouse. The two test groups were compared to the negative controls by gender, separately and combined. Statistical analysis was performed using the qui-square test.

Salmonella Mutagenicity Assay

Strains

The *Salmonella typhimurium* strains TA98 and TA100, as described by MARON & AMES (1983), were kindly provided by Dr. B.N. Ames, University of California, Berkeley, CA (U.S.A.).

Microsonal fraction

The microsonal fraction S9 was prepared from livers of Spregue-Dauley rats pretreated with polychlorinated biphenyl mixture (Aroclor 1254), purchased from Molecular Toxicology Inc. (Moltox™). The S9-mix metabolic activation mixture was prepared according to MARON & AMES (1983).

Salmonella/ microsome assay

Mutagenicity was assayed by the preincubation procedure (MARON & AMES, 1983), with *Salmonella typhimurium* strains TA98 and TA100 using various concentrations of triazoles and hydrazides derivatives with or without S9-mix. The mixture consisting of test compound, 500ml of S9-mix (in tests with metabolic activation) and 100ml of the bacterial suspension (1-2 x 10^9cells/ml), was preincubated for 20 min at 37ºC without shaking. Then, 2000ml of top agar (0.55% agar, 0.55% NaCl, 50mM L-histidine, 50mM biotin, pH 7.4, 45ºC) was added in the test tube and poured onto a Petri dish with minimal agar (1.5% agar, Vogel-Bonner E medium, containing 2% glucose). All assays were carried out in triplicate. After incubation for 48h, colonies (His^+ revelants) were counted and the results were expressed as mutagenic index (MI).

For determination of the cytotoxic effects after preincubation the mixture as indicated above was diluted in phosphate buffer (0.02M, pH 7.4) to obtain a suspension containing 1-2 x 10^3cells/ml. Suitable aliquots of this suspension were plated on nutrient agar (0.8% bacto nutrient broth (Difco), 0.5% NaCl and 1.5% agar) in triplicate. Plates were incubated at 37ºC for 48h before counting the surviving colonies.

Negative (appropriate solvent - water for hydrazides and corn oil for triazoles) and positive (5mg sodium azide in water

per plate for TA100 strain; 0.5mg 4-nitroquinoline 1-oxide (4-NQO) in DMSO per plate for TA98) controls were included in each assay. Aflatoxin B1 (1mg per plate) was used as positive control for the tests with metabolic activation for both strains.

The results were classified positive according to VARGAS et al. (1995), i.e., the number of revertants double the spontaneous frequency accompanied by a reproducible dose-response curve. Significance was evaluated by the SALMONEL software (MYERS et al., 1991). When one of these criteria was not met, the samples were considered to show signs of a negative response. The sample was classified as cytotoxic when cell survival was less than 70% in the cell viability test.

Results

Micronucleus Assay

Based on the toxicity test (Table 1) and recommendations of U.S. Environmental Protection Agency Gene-Tox program, the MTD determined were: (a) VN-3: 2000 mg/kg; (b) ECB-93: 2000mg/kg; (c) ECM-1: 500 mg/kg and (d) ECM-4: 2000 mg/kg. The results for bone marrow toxicity can be seen in the right column of tables 2-5. Mortalities in the test occurred only for 2 groups: ECB-93 and ECM-4. To the compounds VN-3 (48h), ECB-93 (24 and 48h), ECM-1 (24h) and ECM-4 (24 and 48 h) reductions in the PCE:NCE ration were observed. The lowest ratio was 0.4 (ECB-93 - 24h). Evaluation of the induction of MN revealed results that were not statistically different from the negative control for all four substances.

Salmonella Mutagenicity Assay

Tables 6 and 7 show the mutagenic index (MI) in *S.typhimurium* TA98 and Ta100 obtained after triazoles and hydrazides derivatives treatment with and without metabolic activation.

In contrast for the positive control, the products VN-3, ECB-93, ECM-1 and ECM-4, were not mutagenic in *S. typhimurium* TA98 and TA100 strains neither in presence nor in absence of metabolic activation by S9-mix.

Discussion

The drugs VN-3, ECB-93, ECM-1 and ECM-4 neither induce chromosome damage in the mouse bone marrow micronucleus assay nor gene mutations in the Salmonella/microsome assay. The compounds VN-3 (48 h), ECB-93 (24 and 48 h), ECM-1 (24 h) and ECM-4 (24 and 48 h) caused toxicity *in vivo* at the dose level used (500-2000 mg/kg). The PCE reductions observed indicate that VN-3 (48h), ECB-93 (24h and 48h), ECM-1 (24h) and ECM-4 (24h and 48h) cause inhibition in the division and maturation of erythroblasts. This can be explained by the principal mechanism of action of chemotherapeutic drugs, i.e., inhibition of DNA synthesis (SIMS & GUTTERIDGE, 1978; HOWELLS, 1985; CRAIG & STITZEL, 1986; KOROLKOVAS, 1988).

Although some of these compounds show "alert structure", structure-activity relationships in mutagenicity and carcinogenicity (TENNANT & ASHBY, 1991), the result can be explained by mechanism that protect against oxidative degradation of DNA, like antioxidant enzymes and aminoacids (BALAGUER, 1990; CLAYSON et al., 1994).

ECB-93, s-triazole, does not have any structure that can induce damage and did not induce micronuclei, or gene mutation. So, this result confirms that compounds without "alert structure" hardly are mutagens (ASHBY & TENNANT, 1991). The hydrazides ECM-1 and ECM-4, due to the fact that they are isoniazide derivatives and produce hydrazine (-NH-N=CH-), which is keep in

circulation after treatment (BRAUN et al., 1984), these compounds may produce mutagenic effects. ECM-4 also contains the nitro aromatic group, which is an anion radical that can cause damage to the cell. Despite these chemical characteristics, ECM-1 and ECM-4 showed negative results in the micronucleus assay. The ECM-4 molecule has a part that is negatively charged, due to nitro group, but there is also a positively charged group due to pyridine group. Therefore it is possible to explain the negative result by the existence of charge transfer complexes or other kind of associations. A similar situation has been described for panidazole (VOOGD et al., 1992). The other s-triazole, VN-3, contain a nitro aromatic ($-NO_2$) group, that in most cases indicates mutagenic (70%) and/or carcinogenic (86%) activity (SHELBY, 1988; TENNANT & ASHBY, 1991). However are these relations, VN-3 did not demonstrate mutagenic activity, like others drugs: e.g. metronidazole (HEDDLE et al., 1983), pentachloronitrobenzene and 1-nitronaphtalene (ASHBY & TENNANT, 1988). This "alert structure", present in these compounds, can be inactivated biologically by an excretion of this kind of structure and by a fast detoxification in vivo (ASHBY & TENNANT, 1988).

As our results did not indicate genotoxic effects of the test compounds, they enabled the further investigation and evaluation of the pharmacological potential of these products.

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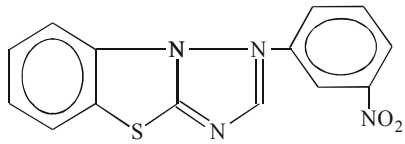
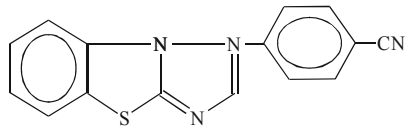
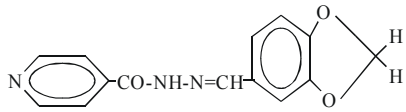
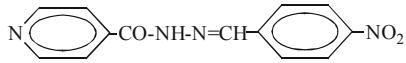
Compound	Chemical name	Chemical structure
benzothiazols derivatives		
VN-3	3-(3'-nitrophenyl)-1,2,4 triazole-[4,3-b]-benzothiazol	
ECB-93	3-(4'-cyanophenyl)-1,2,4-triazole[4,3-b]benzothiazol	
isoniazids derivatives		
ECM-1	4-(3', 4'-methylenedioxybenzilidene)-pyridine carboxilic acid hydrazide	
ECM-4	4-(4'-nitrobenzilidene)-pyridine carboxilic acid hydrazide	

Figure 1. *Nomenclature and chemical structural formula of benzothiazols and isoniazids new derivatives*

Table 1. *Mortality frequency (%) observed to different doses for VN-3, ECB-93, ECM-1 and ECM-4 on four mice tested. * Toxicity signs*

		Mortality with Treatment (mg/kg)		
Substance	**Exposition (h)**	**500 (mg/kg)**	**1000 (mg/kg)**	**2000 (mg/kg)**
VN-3	24	zero	zero	zero
	48	zero	zero	zero
	72	zero	zero	zero
ECB-93	24	zero	zero	zero
	48	zero	zero	zero
	72	Zero	zero	zero
ECM-1	24	Zero*	50	50
	48	Zero	50	50
	72	Zero	zero	zero
ECM-4	24	zero	zero	zero
	48	zero	zero	zero
	72	zero	zero	zero

Table 2. *Number of micronucleated polychromatic erytrocytes (mPCE) of bone marrow of mice treated with VN-3 (2000 mg/kg) and frequency of polychromatic erytrocytes (PCE-%)*

Group	Gender	mPCE				PCE (%)
		Individual (mPCE/2000 PCE)	Per Gender		Per Group	mPCE (‰)	
			Σx	M ± S.D.	M ± S.D.		
Positive	M	102 77 97 93 76	445**	89.0 ± 11.9	88.2 ± 11.9	44.1**	49
Control	F	98 65 93 86 95	437**	87.4 ± 13.3			
Negative	M	5 5 2 1 1	14	2.8 ± 2.0	2.3 ± 1.7	1.2	54
Control	F	3 0 3 1 2	9	1.8 ± 1.3			
Treated	M	3 2 4 5 4	18	3.6 ± 1.1	3.5 ± 1.1	1.8	54
24h	F	3 4 2 3 5	17	3.4 ± 1.1			
Treated	M	4 3 1 1 3	12	2.4 ± 1.3	2.2 ± 1.1	1.1	40
48h	F	3 1 1 2 3	10	2.0 ± 1.0			

*** P<0.001; M ± S.D.= mean ± standard deviation*

Table 3. *Number of micronucleated polychromatic erytrocytes (mPCE) of bone marrow of mice treated with ECB-93 (2000 mg/kg) and frequency of polychromatic erytrocytes (PCE-%).*

Group	Gender	mPCE				PCE (%)
		Individual (mPCE/2000 PCE)	Per Gender		Per Group	mPCE (‰)	
			Σx	M ± S.D.	M ± S.D.		
Positive	M	94 61 92 119 91	457**	91.4 ± 21.0	84.5 ± 20.0	42.3**	46
Control	F	84 96 82 81 45	388**	77.6 ± 19.0			
Negative	M	4 7 6 10 8	35	7.0 ± 2.2	6.9 ± 2.4	3.5	36
Control	F	9 10 7 4 4	34	6.8 ± 2.8			
Treated	M	7 4 12 7 6	36	7.2 ± 3.0	7.4 ± 2.6	3.7	24
24h	F	5 10 8 (?) (D)	23[a]	7.7 ± 2.5			
Treated	M	7 3 7 5 3	25	5.0 ± 2.0	5.3 ± 2.1	2.7	33
48h	F	3 9 6 6 4	28	5.6 ± 2.3			

*** P<0.001; M ± S.D.= mean ± standard deviation; (?)= insuficient number of PCE; (D)= died; [a]= Σ3 animals; [b] =Σ8 animals*

Table 4. *Number of micronucleated polychromatic erytrocytes (mPCE) of bone marrow of mice treated with ECM-1 (500 mg/kg) and frequency of polychromatic erytrocytes (PCE-%).*

Group	Gender	mPCE				PCE (%)
		Individual (mPCE/2000 PCE)	Per Gender		Per Group	mPCE (‰)	
			Σx	M ± S.D.	M ± S.D.		
Positive	M	25 26 16 32 12	118**	23.6 ± 6.3	28.3 ± 8.0	14.2**	67
Control	F	43 23 33 33 33	165**	33.0 ± 7.1			
Negative	M	2 3 1 2 5	13	2.6 ± 1.5	2.1 ± 1.5	1.2	56
Control	F	3 1 1 0 3	8	1.6 ± 1.3			
Treated	M	0 2 1 2 2	7	1.4 ± 0.9	1.5 ± 0.7	0.8	48
24h	F	1 2 2 2 1	8	1.6 ± 0.5			
Treated	M	1 0 2 1 1	5	1.0 ± 0.7	1.2 ± 0.9	0.6	73
48h	F	0 1 3 1 2	7	1.4 ± 1.1			

*** P<0.001; M ± S.D.= mean ± standard deviation*

Table 5. Number of micronucleated polychromatic erytrocytes (mPCE) of bone marrow of mice treated with ECM-4 (2000 mg/kg) and frequency of polychromatic erytrocytes (PCE-%).

Group	Gender	mPCE				PCE (%)
		Individual (mPCE/2000 PCE)	Per Gender		Per Group	mPCE (‰)	
			Σx	M ± S.D.	M ± S.D.		
Positive	M	35 41 33 28 32	169**	33.8 ± 4.8	29.5 ± 5.9	14.8**	55
Control	F	25 29 20 26 26	126**	25.2 ± 3.3			
Negative	M	2 2 2 1 2	9	1.8 ± 0.4	2.2 ± 1.0	1.1	56
Control	F	4 4 2 2 1	13	2.6 ± 1.3			
Treated	M	2 2 3 4 2	13	2.6 ± 0.9	2.9 ± 0.7	1.5	50
24h	F	3 3 4 3 3	16	3.2 ± 0.4			
Treated	M	3 2 1 (D) (D)	6[(a)]	2.0 ± 1.0	2.6 ± 1.0	1.3	32
48h	F	3 3 2 4 (D)	12[(b)]	3.0 ± 0.8			

*** P<0.001; M ± S.D.= mean ± standard deviation; (D)= died; [(a)]= Σ3 animals; [(b)] =Σ4 animals; [(c)] =Σ7 animals*

Table 6. Response to hydrazides (ECM-1 and ECM-4) and s-triazoles (VN-3 and ECB-93) derivatives in the Salmonella/microsome assay, strains TA98 and TA100, without metabolic activation.

	TA98				TA100			
Agent	Dose(µg)	RN	M ± S.D.	MI	Dose(µg)	RN	M ± S.D.	MI
C+	0.5	302, 258, 222	260.7 ± 40.1	9.5	5.0	283, 298, 305	295.3 ± 11.2	2.6
ECM-1	0	16, 13, 10	13.0 ± 3.0	-	0	138, 53, 51	80.7 ± 49.7	-
	1000	16, 18, 19	17.7 ± 1.5	1.4	1000	125, 77, 88	96.7 ± 25.2	1.2
	2500	19, 26, 15	20.0 ± 5.6	1.5	2500	70, 81, 84	78.3 ± 7.4	1.0
	5000	20, 20, 11	17.0 ± 5.2	1.3	5000	145, 131, 50	108.7 ± 51.3	1.3
ECM-4	0	16, 16, 19	17.0 ± 1.7	-	0	125, 64, 63	84.0 ± 35.5	-
	1000	9, 13, 22	14.7 ± 6.7	0.9	1000	118, 108, 95	107.0 ± 11.5	1.0
	2500	20, 17, 19	18.7 ± 1.5	1.1	2500	121, 124, 149	131.3 ± 15.4	1.2
	5000	20, 24, 16	20.0 ± 4.0	1.2	5000	140, 148, 131	139.7 ± 8.5	1.3
VN-3	0	26, 30, 26	27.3 ± 2.3	-	0	103, 127, 110	113.3 ± 12.3	-
	1000	20, 28, 32	26.7 ± 6.1	1.0	1000	106, 162, 96	121.3 ± 35.6	1.1
	2500	35, 27, 29	30.3 ± 4.2	1.1	2500	109, 116, 107	110.7 ± 4.7	1.0
	5000	35, 25, 23	27.7 ± 6.4	1.0	5000	103, 113, 118	111.3 ± 7.6	1.0
ECB-93	0	25, 21, 22	22.7 ± 2.1	-	0	129, 90, 99	106.0 ± 20.4	-
	1000	31, 35, 21	29.0 ± 7.2	1.3	1000	140, 103, 83	108.7 ± 28.9	1.0
	2500	21, 24, 26	23.7 ± 2.5	1.0	2500	110, 136, 102	116.0 ± 17.8	1.1
	5000	26, 30, 30	28.7 ± 2.3	1.3	5000	100, 88, 126	104.7 ± 19.4	1.0

C+= positive control group; RN= revertant number; M ± S.D.= mean ± standard deviation; MI= mutagenic index

Table 7. Response to hydrazides (ECM-1 and ECM-4) and s-triazoles (VN-3 and ECB-93) derivatives in the Salmonella/microsome assay, strains TA98 and TA100, with metabolic activation

	TA98				TA100			
Agent	Dose(µg)	RN	M ± S.D.	MI	Dose(µg)	RN	M ± S.D.	MI
C+	1.0	681, 734, 696	703.7 ± 27.3	22.2	1.0	500, 548, 651	566.3 ± 77.2	3.9
ECM-1	0	34, 64, 37	45.0 ± 16.5	-	0	173, 136, 174	161.0 ± 21.7	-
	1000	44, 32, 29	35.0 ± 7.9	0.8	1000	164, 146, 162	157.3 ± 9.8	1.0
	2500	46, 46, 37	43.0 ± 5.2	0.9	2500	141, 162, 150	151.0 ± 10.5	0.9
	5000	31, 37, 36	34.7 ± 3.2	0.8	5000	154, 167, 178	166.3 ± 12.0	1.0
ECM-4	0	26, 38, 31	31.7 ± 6.0	-	0	169, 146, 126	147.0 ± 21.5	-
	1000	36, 53, 49	46.0 ± 8.9	1.5	1000	150, 143, 171	155.3 ± 15.7	1.1
	2500	39, 37, 51	42.3 ± 7.6	1.3	2500	132, 162, 144	146.0 ± 15.1	1.0
	5000	40, 37, 43	40.0 ± 3.0	1.6	5000	141, 162, 163	155.3 ± 12.4	1.0
VN-3	0	27, 30, 40	32.3 ± 6.8	-	0	157, 152, 287	198.7 ± 76.5	-
	200	34, 38, 44	38.7 ± 5.0	1.2	200	266, 131, 152	183.0 ± 72.6	0.9
	600	44, 26, 35	35.0 ± 9.0	1.1	600	169, 191, 185	181.7 ± 11.4	0.9
	1000	33, 30, 38	33.7 ± 4.0	1.0	1000	183, 163, 164	170.0 ± 11.3	0.9
ECB-93	0	39, 34, 45	39.3 ± 5.5		0	152, 129, 167	149.3 ± 19.1	-
	200	35, 30, 29	31.3 ± 3.2	0.8	200	162, 176, 179	172.3 ± 9.1	1.2
	600	27, 34, 33	31.3 ± 3.8	0.8	600	151, 151, 156	152.7 ± 2.9	1.0
	1000	47, 37, 32	38.7 ± 7.6	1.0	1000	139, 182, 144	155.0 ± 23.5	1.0

C+= positive control group; RN= revertant number; M ± S.D.= mean ± standard deviation; MI= mutagenic index