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Synthesis and mutagenicity of 2-aryl-substitute (*o*-hydroxy-, *m*-bromo-, *o*-methoxy-, *o*-nitro-phenyl or 4–pyridyl) benzothiazole derivatives on *Salmonella typhimurium* and human lymphocytes exposed *in vitro*

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Abstract: Derivatives of 2-aryl-substitute (*o*-hydroxy-, *m*-bromo-, *o*-methoxy-, *o*-nitro-phenyl or 4-pyridyl) benzothiazole were synthesized and tested for their mutagenicity in *in vitro* assays: (i) in the Ames test with *Salmonella typhimurium* TA98 and TA100 strains; and (ii) in the sister chromatid exchange (SCE) in cultured human lymphocytes. The four of compounds (BT-11, B-12, BT-14 and BT-15) caused statistically significant increase in revertant colonies of TA98 and TA100. Treatment of lymphocytes with compounds also caused a significant increase in SCE/cell in association with high levels and long exposure (300 μg/mL and 48 h) of the four compounds. It can be concluded that benzothiazole derivatives showed mutagenic activity and were also able to exert a genotoxic effect reducing both the replication index and mitotic index.

Key words: 2-aryl-substitute benzothiazole; sister chromatid exchange; Ames test.

Abbreviations: BT, benzothiazole; MI, mitotic index; RI, replication index; SCE, sister chromatid exchange.

Introduction

The benzothiazole (BT) ring is present in various natural or synthetic compounds which have useful physiological activities such as neuroprotective (Cao et al. 2002; Ramirez et al. 2003), inhibitor for muscarinic receptor (Jung et al. 2000), Lck enzyme (Das et al. 2003) and also for HIV-protease and reverse transcriptase (Akbay et al. 2003; Nagarajan et al. 2003). Antifungal (Ryu et al. 2003), anti-bacterial (Turan-Zitouni et al. 2003), anti-inflammatory and anti-allergic (Ban et al. 1998) activities have also been reported for several BT derivatives. A series of phenyl BT compounds synthesized as prodrugs were tested for their antitumor activities and selected for clinical evaluation (Hall et al. 1999; Bradshaw & Westwell 2004; Westwell 2004). Among the series, especially quinol and 2-(4-aminophenyl) derivatives of BT have been found as the most effective inhibitors on cancer *in vitro* and *in vivo* (Wells et al. 2000; Hose et al. 2003; Leong et al. 2003; Brantley et al. 2005). Recently, a fluorinated 2-arylbenzothiazole has been reported to have potent and selective inhibitory activity against lung, colon and breast cancer cells (Mortimer et al. 2006). On the other hand, some amino BT derivatives have been suggested to possess also mutagenic potency depending on metabolic activation (Sato et al. 2000; Seo et al. 2000) or by inducing DNA damage (Brantley et al. 2003). In our previous work, we have also synthesized and showed that 2-*m*-tolyl-BT and 2-*p*-tolyl-BT derivatives have mutagenic activity in T100 strain of *Salmonella* (Zeytinoglu et al. 2006).

Therefore the aim of the present study was to synthesize five derivatives of 2-substitue (*o*-hydroxy-, *m*-bromo-, *o*-methoxy-, *o*-nitro-) phenyl or 4-pyridyl BT as very important prodrugs and then, for the first time, to investigate the their eventual mutagenicity by multiple *in vitro* test systems. Gene mutation and other chromosomal aberrations are all involved in cancer development and inherited clinical disorders or anti-tumor activity. To prevent the human exposure to potential genotoxic inducers, it is important to determine such agents in *in vitro* multiple system for mutagenicity. For this purpose, a bacterial Ames test with *Salmonella typhimurium* TA98 and TA100 strains in the presence or absence of rat liver S9 metabolic activation and mammalian assays sister chromatid exchange (SCE) in cultured lymphocytes from human peripheral blood were performed in the presence of the compounds, and then the possible relationship between the position of the substituents and the mutagenicity of these substances were discussed.

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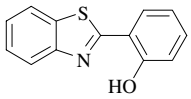
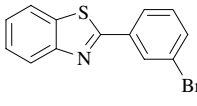
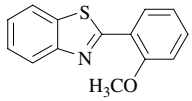
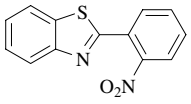
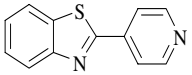
Table 1. Some characteristics of the synthesized 2-aryl-substituted-benzothiazoles.

Compound and R	Molecular formula	Method	Yield [%]	Mw [g/mol]	m.p. [℃] (Exp./Ref.)
BT-11 Ph-*o*-OH	2-(*o*-hydroxyphenyl)-benzothiazole  $C_{13}H_9NOS$	$NaHSO_3$	68	227.273	126/132–3[21]
BT-12 Ph-*m*-Br	2-(*m*-bromophenyl)-benzothiazole  $C_{13}H_8BrNS$	$NaHSO_3$	67	290.174	88
BT-13 Ph-*o*-OCH_3	2-(*o*-methoxyphenyl)-benzothiazole  $C_{14}H_{11}NOS$	$NaHSO_3$	75	241.301	98/105–6 [21]
BT-14 Ph-*o*-NO_2	2-(*o*-nitrophenyl)-benzothiazole  $C_{13}H_8N_2O_2S$	PPA	73	256.278	84[24]
BT-15 4-Pyridyl	2-(4-pyridyl)-benzothiazole  $C_{12}H_8N_2S$	PPA	82	212.270	120/163–7[28], 135–6[21]

Material and methods

The synthesis of compounds

Five derivatives of 2-aryl-substituted-BT (Table 1) were synthesized by the condensation reactions which were carried out by reacting *o*-aminothiophenol as free amine and aldehyde or carboxylic acid. Cyclization agents HCl, PPA or PPE were used in the acid reaction (Zeytinoglu et al. 2006). Equimolar amounts of *o*-aminothiophenol (0.015 M) and carboxylic acid derivative were mixed and heated in a suitable dehydratation agent, and then stirred for 1–2 hours at 100–110 °C. Following TLC monitoring ($CHCl_3$:MeOH, 98:2), reaction vessel was poured onto the mixture of ice-water and neutralized with saturated Na_2CO_3 solution, and then filtered. Finally, it was dried on air and re-crystallized from EtOH, after washing with water.

The mixture of equimolar amount of $NaHSO_3$ and aldehyde (0.012 M) was refluxed in EtOH (15 mL) for 15–20 min in the aldehyde reaction. Amine derivative (*o*-aminothiophenol) (0.01 M) was added and continued to reflux for 4–5 h. TLC monitoring was performed in $CHCl_3$:MeOH (98:2) solvent system. After cooling, EtOH was evaporated from the solution under the reduced pressure, washed with water, filtered and then dried. Finally it was purified by re-crystallization from EtOH (Isikdag & Ucucu 1990).

Except for one of the synthesized compounds, 2-(*m*-bromo-phenyl)-benzothiazole (BT-12), all the compounds were reported on in the literature (Hides & Hankovsky 1963; Isikdag & Ucucu 1990; Jung et al. 2000; Ikegami & Arai 2002; Ulrich 2002; Chakraborti et al. 2004; He et al. 2004; Kodomari et al. 2004; Matsushita et al. 2004). BT-12 was the compound synthesized originally within this study.

Equipment and analysis of the compounds

The structures of the compounds were elucidated by EI-MS, IR and ^{1}H-NMR spectroscopic methods. EI-MS were recorded on a VG Zabspec mass spectrometer using reagents (Sigma-Aldrich, Merck, Fluka) without purification. Melting points of the compounds were determined by an Electrothermal 9100 Model melting point apparatus and reported uncorrected. TLC systems for routine monitoring of reaction mixtures, and confirming the homogeneity of analytical samples, used Kieselgel $60F_{254}$ (0.25 mm) silica gel TLC aluminum sheets (Merck) and spots were visualized by UV lamp (CAMAG CE 12VDC/VAC 50/60 Hz at 254/366 nm).

IR spectra were recorded on a JASCO FT-IR-430 spectrophotometer (KBr; ν_{max} in cm^{-1}). ^{1}H-NMR (400 MHz) spectra were recorded at rt using a Bruker (400 MHz) NMR spectrometer with tetramethylsilane as internal standard. Chemical shifts (δ) are reported in ppm, relative to solvent peaks for DMSO-d_6, (δ 2.50 and δ_{TMS} 0 for ^{1}H) and coupling constants (J) values are given in Hertz. Multiplicities are reported as singlet (s), doublet (d), triplet (t), multiplet (m), broad (br), and overlapping (ov).

Ames assay

The standard plate incorporation test was performed according to Maron & Ames (1983) using *Salmonella typhimurium* strains TA98 and TA100. The test compounds at different concentrations (100, 500, 1000, 2500 and 5000 μg/plate) dissolved in DMSO were tested for mutagenicity. Three plates at two separate experiments were used for each concentration tested and for positive and negative controls. The revertant colonies on all plates were counted after 48 h of incubation.

Mutagenic activity of the compounds in the presence of metabolic activity was investigated by addition of rat liver homogenate (S9 fraction). The S9 fraction was prepared from rats after six days injected intraperitoneally with methylcholantren (80 mg/kg, Sigma), and stored at −80 °C. The S9 mixture was prepared by mixing rat liver (15%) and mutagenicity test tablets containing NADP and glucose-6-phosphate following the method of manufacturer (Boehringer Mannheim Biochemicals, Germany).

Three positive controls; in the absence of S9, 4-nitro-*o*-phenylenediamine (4-NPD, 20 μg/plate) and sodium azide (NaAz, 1.5 μg/plate) for TA98 and TA100, respectively, aminofluorene (2-AF, 10 μg/plate) for all strains with S9, and solvent control were run concurrently with all experiments.

Sister chromatid exchange in human lymphocytes

Lymphocyte cultures were set up by adding heparinized peripheral blood (0.5 mL) obtained from two young, non-smoking, healthy donors, into Chromosome Medium B (Seromed Biochrom) containing phytohaemagglutinin (PHA, 1%) and incubated for 72 h at 37 °C. For SCE assay, 5-bromo-2-deoxyuridine (BrdU, 10 μg/mL, Sigma) was added at the initiation of the cultures, and then test compounds were added after 24 h incubation of cultures. The cultures were directly exposed to the test compounds (75, 150 and 300 μg/mL) dissolved in DMSO. DMSO (20 μL/mL) and mitomycin C (MMC, 1 μg/mL) were used as solvent and positive controls, respectively. The cultures were incubated for an additional 24 and 48 h at 37 °C and colchicine (0.06 μg/mL) was applied 2 h prior to harvest. Metaphase cells were re-suspended in KCl (0.4%) after centrifugation and fixed in cold MeOH-acetic acid (3:1, v/v). Slides were stained using Giemsa technique (Benn & Perle 1992). Two experiments were carried out for each treatment.

Analysis of SCE was performed in M2 metaphases with a minimum 50 in all satisfactorily spreading and stained mitosis (25 cells from each donor) per point. The metaphases were selected on the basis of good morphology and intact appearance. The replication index (RI) was determined by scoring metaphase cells in 100 cells of each metaphase 1, metaphase 2 and metaphase 3 and by the formula of RI = 1×(M1) + 2×(M2) + 3×(M3) / 100. The statistical significance for all individual treatments was determined by using ANOVA Dunnet's test.

Results and discussion

Analysis of synthesized 2-aryl-benzothiazole compounds

Reported compounds were synthesized in a straightforward manner from easily available precursor *o*-aminothiophenol, and their structural assignments were established by IR, ^{1}H-NMR and EI-MS spectroscopic investigations. Some characteristics of synthesized compounds such as reaction time, yield and melting point are shown in Table 1.

The pharmaceutical interest of the BT skeleton may be limited because of the character of substituents, such as amino, nitro, alkyl or aryl groups. The synthetic route described in this paper is limited with one-step reaction. The interest of the multi-step synthesis would allow more active compounds following further modulations of the ring in various positions. But with this study, we focused on the preliminary genotoxicity of basic aza analogs such as BTs.

According to IR analysis, significant stretching bands due Ar C-H were 3148–3003 cm^{-1}, C$\bar{N}$ and C$\bar{C}$ were 1640–1500 $cm^{-}1$ for all compounds. ^{1}H-NMR spectra for all compounds were recorded at 400 MHz by using DMSO-d_6 as solvent. Results were found as follows:

BT-11 as 11.63 (1H, bs, OH), 8.16 (2H, dd, J = 7.84, 7.95), 8.06 (1H, d, J = 8.12), 7.55 (1H, t, J = 7.63), 7.43 (2H, m), 7.09 (1H, d, J = 8.23), 7.01 (1H, t, J = 7.53);

BT-12 as 8.31 (1H, s), 8.26 (1H, s), 8.18 (1H, d, J = 8.09), 8.08 (2H, t, J = 3.88), 7.79 (1H, d, J = 8.07), 7.57 (1H, m), 7.51 (1H, m);

BT-13 as 8.44 (1H, d, J = 7.84), 8.13 (1H, d, J = 8.12), 8.05 (1H, d, J = 8.08), 7.55 (2H, m), 7.44 (1H, t, J = 7.56), 7.31 (1H, d, J = 8.41), 7.17 (1H, d, J = 7.57), 4.07 (3H, s, OCH_3);

BT-14 as 8.24 (1H, d, J = 7.88), 8.09 (1H, d, J = 7.87), 8.06 (1H, d, J = 7.96), 7.99 (1H, d, J = 7.48), 7.86 (2H, m), 7.57 (2H, m); and

BT-15 as 8.79 (2H, d, J = 4.85), 8.23 (1H, d, J = 7.95), 8.15 (1H, d, J = 8.06), 8.03 (2H, d, J = 4.88), 7.57 (2H, m).

Mutagenic activity of the compounds

Ames assay provides a very sensitive study of potentially mutagenic pathways for the metabolism of chemicals in both the absence and the presence of a rat liver microsomal system (S9 mix). Increasing doses of these compounds were tested using most sensitive *Salmonella* strains TA98 and TA100, and compared with the results of solvent control (DMSO) for in both the absence and the presence of a rat liver microsomal system (S9 mix). Positive results were represented as an increase in the numbers of revertant colonies. Toxicity of these chemicals to bacteria was observed on the basis of the significantly reduced number of revertant compared to solvent control. In the each experiment set, a suitable known mutagen was tested as a positive control and the results of the test are given in Table 2.

Results showed that four out of the five substances (BT-11, BT-12, BT-14 and BT-15) caused statistically significant ($p < 0.001$) increase in revertant colonies when compared with the solvent control. BT-14 was most mutagenic one especially for TA98 ($p < 0.001$) in the absence of S9, whereas it was less mutagenic for TA100 ($p < 0.01$). Two high doses of BT-15 and BT-12 showed strong mutagenic activity only for TA98 ($p < 0.001$) while BT-11 was mutagenic for TA100. BT-13 showed least mutagenic activity only at highest dose (5000 μg/plate). The positive control compounds 4-NPD, 2-AF and NaAz showed very high frequencies of revertant colonies over those of solvent control and treated cultures. Mutagenic activity of most doses of chemicals was increased a dose-related manner.

Salmonella strains used in this work have different type mutations in their genome. The strain TA98 contains a frame-shift mutation and the TA100 strain con-

Table 2. Number of revertants induced by 2-aryl-substituted-benzothiazoles in Ames *Samonella*/microsome test with or without metabolic activity.

Chemicals	Doses [μg/mL]	Number of revertants/plate[a]			
		TA98		TA100	
		S9 (-)	S9 (+)	S9 (-)	S9 (+)
BT-11	5000	32 ± 4**	36 ± 0	99 ± 5***	113 ± 6***
	2500	27 ± 1	29 ± 3	97 ± 1**	106 ± 6***
	1000	25 ± 4	30 ± 1	82 ± 0	101 ± 4**
	500	26 ± 2	26 ± 3	77 ± 1	90 ± 7
	100	18 ± 2	21 ± 1	61 ± 5	90 ± 2
BT-12	5000	36 ± 2***	38 ± 1	96 ± 2**	105 ± 5**
	2500	33 ± 4**	34 ± 1	80 ± 5	96 ± 8
	1000	25 ± 3	31 ± 3	78 ± 3	86 ± 6
	500	19 ± 3	22 ± 2	62 ± 4	82 ± 2
	100	22 ± 1	23 ± 1	60 ± 3	75 ± 3
BT-13	5000	31 ± 1*	35 ± 2	90 ± 2	99 ± 5**
	2500	23 ± 3	27 ± 2	69 ± 3	92 ± 2
	1000	22 ± 2	26 ± 3	69 ± 1	84 ± 5
	500	21 ± 2	22 ± 2	67 ± 4	76 ± 4
	100	18 ± 2	24 ± 1	55 ± 3	75 ± 1
BT-14	5000	84 ± 2***	89 ± 0***	95 ± 4**	96 ± 0**
	2500	73 ± 1***	81 ± 1***	87 ± 1	93 ± 0*
	1000	45 ± 2***	60 ± 1***	83 ± 3	89 ± 4
	500	29 ± 3	30 ± 0	81 ± 1	82 ± 2
	100	23 ± 1	24 ± 2	72 ± 2	69 ± 4
BT-15	5000	47 ± 2***	58 ± 2***	71 ± 3	91 ± 1
	2500	38 ± 2***	42 ± 1*	60 ± 1	87 ± 5
	1000	24 ± 4	38 ± 1	75 ± 4	78 ± 7
	500	20 ± 4	29 ± 1	76 ± 4	80 ± 2
	100	22 ± 3	26 ± 1	77 ± 6	77 ± 5
DMSO	100	23 ± 4	32 ± 1	79 ± 3	81 ± 3
Spontaneous	–	22 ± 1	27 ± 1	88 ± 3	93 ± 2
4–NPD	2	470 ± 3***	–	–	–
2-AF	10	–	625 ± 3***	–	1036 ± 4***
NaAz	1.5	–	–	895 ± 2***	–

[a] (–) S9 = without and (+) S9 = with metabolic activation. The results are mean and ± S.D. of five or six plates. Positive controls: 2-AF = 2-aminofluorene, NaAz = sodium azide and 4-NPD = 4-nitro-*o*-phenylenediamine. Negative control: DMSO = dimethylsulphoxide [μL/mL]. A result of each concentration was compared with the solvent control by Dunnet's test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

tains a base-pair substitution (Maron & Ames 1983). Therefore, differences in the activity of a compound acting in these strains may yield some insight into how the compounds interact with DNA of bacteria. Three of these compounds (BT-12, BT-14 and BT-15) generally reverse TA98 independent of S9, and regarded as direct mutagens base-pair substitution rather than frame-shift. We can say that BT-11 and also others, excluding BT-15, exhibit a mutagenic activity as direct mutagens frame-shift rather than base-pair substitution. In addition, some of the substances increased or decreased the number of revertant colonies at only single dose, such as BT-11 on TA98 without S9; BT-12 on TA100 and BT-13 on TA98 and TA100. They were taken as a false response since effects of the other doses were meaningful.

The features of lymphocytes exposed to compounds and the mean SCE/cell in these groups are summarized in Table 3. A significant increase ($p < 0.05$) in SCE/cell was observed in association with high levels and long exposure (300 μg/mL and 48 h) of all compounds except BT-12. No statistically significant increases in SCE were observed at any of the doses tested for these compounds for 24-h exposure compared to the controls. Significant differences were observed, as expected, in SCE with the positive control MMC (0.1 μg/mL).

In order to determine the RI and MI for each doses of the compounds, metaphase cells in mitosis 1, 2 and 3 were scored as indicated in the Materials and methods. In all of five 2-aryl-substituted-benzothiazole, especially in all doses of BT-13 and BT-15, values of RI and MI were significantly decreased ($p < 0.01$) similar to positive control level (Table 3). BT-12 was relatively less effective than others on the inhibition of replication and mitosis. The positive control compounds MMC showed very high inhibition rate of RI and MI over those of solvent control and treated cultures.

The decreased level of replication and mitosis rate for 2-aryl-substituted-benzothiazoles supports the find-

Table 3. Frequency of SCE, RI and MI in human lymphocytes exposed to 2-aryl-substituted- benzothiazoles for 24 and 48 h.[a]

Compounds [μg/mL]	SCE/cell (mean ± SD)		M1	M2	M3	RI ± SD	MI ± SD
	24 h	48 h				48 h	
BT-11							
300	6.4 ± 0.6	7.2 ± 0.4*	64	32	4	1.4 ± 0.1*	1.3 ± 0.2*
150	6.0 ± 0.5	6.8 ± 0.6	56	37	7	1.5 ± 0.7*	1.4 ± 0.3*
75	5.3 ± 0.4	6.5 ± 0.3	49	43	8	1.6 ± 0.5	1.9 ± 0.4
BT-12							
300	6.2 ± 0.6	7.0 ± 0.6	58	37	5	1.4 ± 0.3*	1.5 ± 0.3*
150	5.9 ± 0.6	5.5 ± 0.7	54	38	7	1.5 ± 0.4*	1.8 ± 0.4
75	5.8 ± 0.7	5.3 ± 0.5	41	53	6	1.6 ± 0.7	2.3 ± 0.4
BT-13							
300	6.8 ± 0.6	7.8 ± 0.3*	72	21	7	1.3 ± 0.1*	0.9 ± 1.0**
150	6.3 ± 0.7	6.4 ± 0.8	62	33	5	1.4 ± 0.3*	1.2 ± 0.4**
75	6.0 ± 0.4	5.8 ± 0.7	60	34	6	1.4 ± 0.8*	1.7 ± 0.2*
BT-14							
300	5.9 ± 0.5	7.3 ± 0.4*	74	22	4	1.3 ± 0.4*	1.4 ± 0.3*
150	5.8 ± 0.5	6.9 ± 0.6	67	27	6	1.4 ± 0.5*	1.7 ± 0.2*
75	5.4 ± 0.7	5.9 ± 0.7	55	38	7	1.5 ± 0.6*	2.6 ± 0.3
BT-15							
300	6.7 ± 0.6	7.0 ± 0.5*	69	26	5	1.3 ± 0.5*	0.8 ± 0.3**
150	6.1 ± 0.3	6.4 ± 0.8	63	29	8	1.4 ± 0.7*	1.2 ± 0.4**
75	5.6 ± 0.7	5.2 ± 0.6	52	38	10	1.5 ± 0.6*	1.6 ± 0.2*
DMSO [20]	4.9 ± 0.6	5.5 ± 0.6	23	29	48	2.2 ± 3.0	3.1 ± 0.2
MMC [0.1]	12.3 ± 0.7***	17.2 ± 0.9***	79	18	3	1.2 ± 0.5*	1.2 ± 0.3**
Spontaneous	4.8 ± 0.6	ND	18	31	51	2.3 ± 0.4	3.5 ± 0.4

[a] A total of 50 second metaphases of each group were scored for SCE and 100 for RI and MI. DMSO = dimethylsulphoxide in [μL/mL], MMC = mitomycine C, ND = not done, M1 = metaphase 1, M2 = metaphase 2, M3 = metaphase 3, SD = standard deviation, *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ (Dunnet's test).

ing of those reported anti-tumor activities and IC_{50} values at nanomolar level of some other BT derivatives (Shi et al. 1996; Hall et al. 1999; Wells et al. 2000; Bradshaw et al. 2001; Hutchinson et al. 2003). We have also shown in a previous work (Tuylu & Zeytinoglu, 2004) that the present compounds slightly increased the number of chromosome aberration and decreased both the RI and MI.

BT-13 less mutagenic for *Salmonella* was found to be mutagenic for human lymphocytes by SCE. On the other hand, BT-12 which was mutagenic in bacterial system did not caused SCE at all. Others, BT-11, BT-14 and BT-15 showed mutagenic activity in all system. In correlation to their mutagenicity, chemicals inhibited replication and mitosis nearly at all doses, including BT-13 which is the least mutagenic one. This inhibition of replication and mitosis may be due to interaction of chemicals with DNA. BT-11 and BT-13 must be further tested before any use in drug preparation, because of their conflicting results in this work. BT-12, BT-14 and BT-15 were mutagenic but may be tested for their activity against cancer cells.

In the present work, mutagenicity of 2-aryl-substituted-benzothiazole derivatives show some similarity to the report by Lefevre et al. (1997) and Sato et al. (2000) that indicated the mutagenic activity of some other BTs. However, 6-aminobenzothiazole exhibited only S9-dependent mutagenicity in Y61029 strain of T100 (Sato et al. 2000) similar to BT-11. Presence of different substitute in BT changed the DNA interaction of the molecule. Interaction of BT with DNA molecule itself has been shown to affect the template activity which inhibits RNA synthesis (Hall et al. 1999). Furthermore, another derivative of BT, 6-(*p*-dimethylaminophenylazo)-benzothiazole was mutagenic in rat livers, producing malignant liver tumors (Lefevre et al. 1997). In our previous work, we have also obtained similar positive results from other different derivatives of BT, as 2-*m*-tolyl- and 2-*p*-tolyl-benzothiazole with a phenyl rings possess methyl group inducing significant mutagenic activity in TA100 (Zeytinoglu et al. 2006). However, some opposing effect of derivatives which bearing the same group on different locations was significant comparing with our previous work. BT-14 (2-(*o*-nitrophenyl)-benzothiazole) exhibited strong mutagenicity while 2-(3-nitrophenyl)-benzothiazole was very toxic. Further, BT-15 (2-(4-pyridyl)-benzothiazole) was found to be more mutagenic in the absence of S9 compared with 2-(2-pyridyl)-benzothiazole. The biological activity of these two BT derivatives indicates that the locations of nitro and pyridyl groups on phenyl ring may affect the activity of compounds. Here, for the first time, compounds were also tested for their possible mutagenicity in a mammalian test system.

Briefly, the chemistry performed herein constitutes simple and one-pot synthesis methods for the condensation of 2-aminothiophenols with aryl alde-

hydes or benzoic acids in the presence of $NaHSO_3$ or HCl/PPA/PPE, respectively. In the cases where aryl aldehydes were employed, the sodium bisulphate adducts were obtained which reacted with o-aminothiophenol to give 2-aryl-substituted-benzothiazole. This is the first report on the mutagenicity of these five 2-aryl-substituted-benzothiazole derivatives. We have performed a comprehensive study of different genotoxicity assay. From these studies it may be concluded that four out of the five BTs are directly acting mutagens for S. *typhimurium* strains, and all BTs are weakly capable inducing SCE at the highest dose after 48-h exposure only. However, these effects of the tested compounds are not showing any structural patterns for their activity. We can only say that a hydroxyphenyl-bound form of BT is much less mutagenic than others. Effects of the tested compounds on chromosome aberration must be considered and they must be tested further.

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