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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A study on the genotoxic activities of some new benzoxazoles

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Abstract The *Bacillus subtilis rec* assay has been specially developed to detect DNA-damaging potential in chemicals, with the rationale based on the relative difference of survival of a DNA repair combination proficient strains and its deficient strain, which is interpreted as genotoxicity. The genotoxic activities of newly **(1–6)** and previously **(7–18)** synthesized various benzoxazoles and benzimidazoles were analyzed via the *B. subtilis rec* assay. Newly obtained benzoxazole and benzimidazole derivatives **(1–6)** were synthesized in the presence of polyphosphoric acid (PPA) and 6 N HCl, respectively to detect their DNA-damaging activities. Among the tested compounds, 6-methyl-2-(*o*-chlorophenyl)benzoxazole **(9),** 5-amino-2-(*p*-methylbenzyl)benzoxazole **(4)**, 5-(*p*-fluorobenzamido)-2-phenylbenzoxazole **(13)**, and 2-(*p*-methylaminophenyl)benzoxazole **(18)** showed genotoxic activities having Rec_{50} values of 1.85, 1.74, 1.60, and 1.50 or S-probit values of 0.534, 0.482, 0.460, and 0.357, respectively. On the other hand, 2-(*p*-bromobenzyl)-5-methylbenzimidazole **(6)** and 2-benzyl-5-(*p*-fluorophenylacetamido)-benzoxazole **(15)** were exhibited a reverse effect that displayed a bacterial growth in the rec^- strains while there was no any bacterial growth in rec^+ strains at the same concentration.

Keywords *Bacillus subtilis rec* assay · Benzimidazoles · Benzoxazoles · DNA-damaging activity · Genotoxic activity

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Introduction

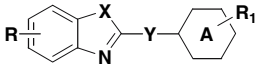
A number of anticancer chemotherapeutic agents are bifunctionally reactive and therefore can crosslink biological macromolecules, including DNA (Reddy and Vasquez, 2005). The ability of these DNA interstrand crosslinks to interfere with DNA trancription and replication and ultimately to cause cytotoxicity appears to be essential for anticancer activity. Both intra- and interstrand DNA crosslinks are formed by some of these agents, as well as DNA-protein crosslinks, all of which may contribute to their cyctotoxicity and chemotherapeutic efficacy. DNA–binding drugs such as cisplatin are components of treatment regimens for various cancer types (Chang *et al.*, 1987; Huerta *et al.*, 2003) Several mechanisms have been implicated in cisplatin resistance, including reduced drug uptake, increased cellular thiol levels, increased or faulty DNA repair mechanisms, and a reduced level of apoptosis (Siddik, 2003).

Genotoxic agents are affecting nucleic acids and alter their function that may directly bind to DNA or they may indi*rec*tly lead to DNA damage by affecting enzymes involved in DNA replication (http://www.Cancerquestorg/index-cfm?page0482). If, DNA damage is severe enough, it will induce cells to undergo apoptosis, the equivalent of cellular suicide. The selectivity of the drug action is based on the sensitivity of rapidly dividing cells, such as cancer cells. Rapidly dividing cells are particularly sensitive to genotoxic agents because they are actively synthesizing new DNA. However, the genotoxic drugs affect both normal and cancerous cells, so that the genotoxicity of these drugs is one of their most serious side effects owing to the possibility of inducing secondary malignancies.

The substituted benzoxazole and benzimidazole derivatives have been the aim of many researches for many years because they constitute an important class of heterocyclic compounds exhibiting substantial antibacterial, antifungal, antitumor, and antiviral activities (Hubschwerlen *et al.*, 1992; Olsen *et al.*, 1994; Staszewski *et al.*, 1995; Kim *et al.*, 1996, 1997; Perrin *et al.*, 1996; Shi *et al.*, 1996; Zhou and Skibo, 1996; Pinar *et al.*, 2004; Plemper *et al.*, 2004; Yildiz *et al.*, 2004). A series of 5-formyl-, 5-(aminocarbonyl)-, or 5- and 6-nitro derivatives of 2-(4-methoxyphenyl)benzimidazoles and benzoxazoles were determined as topoisomerase I inhibitors (Kim *et al.*, 1996). Moreover, substituted pyrimido[1,6-*a*]benzimidazoles were also found as a new class of potent DNA gyrase inhibitors; however, their antibacterial activity was inferior to the quinolone type antibacterial agents such as norfloxacin or fleroxacin (Hubschwerlen *et al.*, 1992). Although benzoxazoles and benzimidazoles are the structural isosters of natural nucleotides and interact easily with the biopolymers, there were no genotoxic studies performed to date showing that these compounds may directly bind to DNA or may indirectly lead to DNA damage by affecting enzymes involved in DNA replication.

In previous studies, we reported some derivatives of benzoxazoles, benzimidazoles, and related fused heterocyclic compounds exhibiting antimicrobial (Yildiz *et al.*, 2004), antiviral (Plemper *et al.*, 2004), and multidrug resistance cancer cell activities (Lage *et al.*, 2006), with inhibiting activity on eukaryotic topoisomerase II enzyme in a cell-free system (Pinar *et al.*, 2004). In this study, a series of benzoxazole and benzimidazole derivatives **1–6** were synthesized and tested for

their genotoxic activity together with the previously synthesized analogs **7–18** (Sener *et al.*, 1991, 1997; Temiz *et al.*, 1998; Aki-Sener *et al.*, 2000; Yildiz *et al.*, 2004; Yildiz-Oren *et al.*, 1997, 2004) (Formula 1) by using the *B. subtilis rec* assay including their structure–activity relationship (SAR) studies. The goal of this study was to determine whether the synthesized compounds have any DNA-damaging genotoxic activity or not, which could be the mode of their chemotherapeutic action.



X= O, NH

Y= —, CH_2, CH_2O

A= C_6H_{11}, C_6H_5, C_6H_4

R= H, 5-Cl, 5-CH_3, 5-NH_2, 5-NO_2, 6-CH_3, 6-NO_2, 5-*p*-fluorobenzamido, 5-(*p*-fluorophenyl)acetamido, 5-(*p*-bromophenyl)acetamido

R_1= H, 2-Cl, 2-NO_2, 4-F, 4-Cl, 4-Br, 4-NH_2, 4-C_2H_5, 4-$NHCH_3$, 4-CH_3, 4-OC_2H_5

Formula 1

Materials and Methods

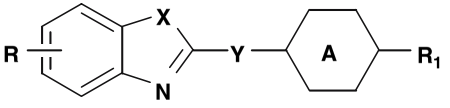
Chemistry

Silicagel HF_{254} chromatoplates (0.3 mm) were used for thin-layer chromatography (TLC). The solvent systems were chloroform–methanol (15:0.5) for compounds **1–6**. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded via Pye Unicam SP-1025 with KBr discs. ^1H nuclear magnetic resonance (NMR) spectra were obtained via a Bruker AC 400 MHz spectrometer in d-chloroform or d_6-dimethyl sulfoxide, and tetramethylsilane (TMS) was used as an internal standard. Mass analyses were carried out with a Fisions-Instruments VG Platform II mass spectrometer using electron ionization. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus. The results of the elemental analyses (C, H, N) were within ± 0.4% of the calculated values. Physical and spectral data of the synthesized compounds are reported in Table 1.

General procedure for the synthesis of benzoxazole derivatives **(1–5)**

A mixture of 4-substituted-2-aminophenols (0.01 mol) and appropriate acids (0.015 mol) was heated over 100°C in polyphosphoric acid (PPA, 12 g) with stirring for 2.5 h according to Yildiz *et al.* (2004). At the end of the reaction period, the residue was poured into an ice-water mixture and neutralized with excess 10% NaOH solution

Table 1 Physical properties and spectral data of the synthesized compounds **1–6**



Compound no.	R	R_1	X	Y	A	Formula	MP (°C)	Yield (%)	IR(cm^{-1})	^{1}H-NMR(δ ppm, J=Hz)	MASS m/e
1	5-Cl 6-NO_2	H	O	—	C_6H_{11}	$C_{13}H_{13}O_3N_2Cl$	88	62	3098, 2936, 1606, 1531, 1448, 1333, 1255, 1013, 994–571	8.05 (1H, s), 7.78 (1H,s), 3.03–2.97 (1H, m), 2.19–2.15 (2H, m), 1.90–1.85 (2H, m), 1.77–1.65 (3H, m), 1.49–1.31 (3H, m)	281(% 100)(M^+)
2	5-NO_2	OC_2H_5	O	CH_2	C_6H_4	$C_{16}H_{14}O_4N_2$	158	38	2976, 1611, 1514, 1451, 1339, 1244, 1018, 952–540	8.14–8.11 (1H,s), 7.31–7.29 (2H, d, J = 8.8), 7.26–7.24 (1H, d, J = 8.8), 6.94–6.92 (2H, d, J = 8.8), 6.89–6.87 (1H, d, J = 8.8), 4.23 (2H, s), 4.06–4.00 (2H, q), 1.37–1.33 (3H, t)	298 (%100)(M^+)
3	5-NH_2	F	O	CH_2	C_6H_4	$C_{14}H_{11}ON_2F$	77	45	3382, 1599, 1563, 1453, 1355, 1300, 1223, 1014, 964–497	7.38–7.32 (2H, m), 7.25–7.22 (1H, d, J = 8.5), 7.05–7.0 (2H, m), 6.96–6.95 (1H, d, J = 2.21), 6.70–6.65 (1H, dd, J = 8.5, J' = 2.24), 4.20 (2H, s)	243(%)(M+1)
4	5-NH_2	CH_3	O	CH_2	C_6H_4	$C_{15}H_{14}ON_2$	85	41	3382, 2917, 1615, 1556, 1453, 1355, 1272, 1021, 960–549	7.32–7.15 (5H, m), 6.98–6.95 (1H, d, J = 2.1), 6.67–6.64 (1H, dd, J = 8.5, J' = 2.19), 4.20 (2H, s), 2.35 (3H, s)	239(%)(M+1)

Table 1 continued

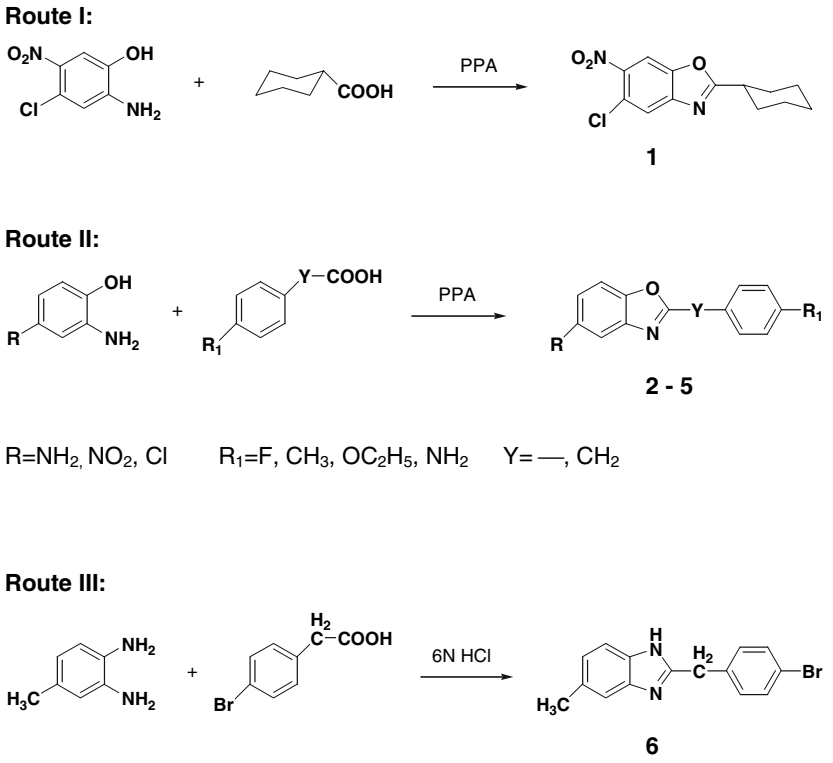
Compound no.	R	R_1	X	Y	A	Formula	MP (°C)	Yield (%)	IR(cm^{-1})	^{1}H-NMR(δ ppm, J=Hz)	MASS m/e
5	5-Cl	NH_2	O	–	C_6H_4	$C_{13}H_9N_{2O}Cl$	197	45	3471, 3327, 1608, 1556, 1458, 1334, 1256, 944–535	7.84–7.81 (2H, d, J = 8.8), 7.72–7.71 (1H, d, J = 2.0), 7.68–7.66 (1H, d, J = 8.4), 7.32–7.30 (1H, dd, J = 8.4, J' = 2.4), 6.67–6.65 (2H, d, J = 8.8), 6.05 (2H, s)	245(%100)(M^+)
6	5-CH_3	Br	NH	CH_2	C_6H_4	$C_{15}H_{13}N_2Br$	131	32	2918, 1630, 1553, 1445, 1178, 1012, 933–576	7.45–7.42 (1H, m), 7.38–7.36 (2H, d, J = 8.8), 7.28 (1H, s), 7.21–7.19 (1H, d, J = 8.4), 7.14–7.12 (2H, d, J = 8.0), 7.10–7.06 (1H, d, J = 8.0), 4.20 (2H, s), 2.40 (3H, s)	301(%100)(M^+)

extracted with benzene; the benzene solution was dried over anhydrous sodium sulfate and evaporated under diminished pressure. The residue was boiled with 200 mg of charcoal in ethanol and filtered and the solvent was removed with a rotary evaporator. The residue was purified via recrystallization from ethanol–water. The obtained needles are dried *in vacuo* (Scheme 1, routes I and II).

5-Methyl-2-(*p*-bromobenzyl)benzimidazole **(6)**: A mixture of *p*-bromophenylacetic acid (0.01 mol) and 4-methyl-2-phenylendiamine (0.01 mol) was boiled under reflux with stirring for 4 h in 15 ml of 6 N HCl according to Sener *et al.* (1997). At the end of the reaction period, the mixture was neutralized with excess $NaHCO_3$. The collected precipitate washed with water and dried *in vacuo*. The residue was purified by column chromatography, eluted with $CHCl_3$, and the obtained product was recrystallized from ethanol–water and needles were dried *in vacuo* (Scheme 1, route III).

Genotoxic study

The *rec* assay was developed for screening chemical and environmental mutagens. Recombinationless mutant cells of *B. subtilis* (M45) are more sensitive to the cell-



Scheme 1 General synthesis of compounds 1–6

killing action of chemical mutagens, e.g., mytomycin C, *N*-nitroso-*N*-methylurethane, etc., than are the wild-type bacteria (H17). The assay is also useful for prescreening anticancer drugs such as dynemicines. Because the sensitivity of the *rec* assay to chemicals having induction activity of DNA damage is higher than that of other screening systems, this method may be useful for prescreening bioactive compounds in crude drugs as well as in microorganisms (Nomura *et al.*, 2002).

Both bacterial strains *B. subtilis* H17 (arg^-, trp^-, $recE^+$) and B. subtilis M45 (arg^-, trp^-, $recE^-$) were obtained from National Institute of Genetics, Mishima, Shizuoka-Ken, Japan. The test consists of comparing the highly sensitive rec^- (*B. subtilis* M45) strain with the wild-type rec^+ (*B. subtilis* H17), the rec^- strain being deficient in recombinant repair. The use of spores is preferred over vegetative cells as spores increase the sensitivity of the assay by 15–20 times over the germinating phase (Kada *et al.*, 1980). The induction of DNA damage and the efficacy of mutation induction were correlated via screening of positive mutagen agent, 4-nitroquinoline 1-oxide (4-NQO). In addition, the antineoplastic agent cisplatin was used as a standard drug. The strains were checked routinely for ultraviolet-light sensitivity and arginine and tryptophan requirements and stored at –80°C.

Preparation of spores

The spores were prepared by spreading overnight broth cultures of the strains in different sets of sterile disposable Petri plates on modified Schaeffer's agar medium (Sharma and Sobti, 2000). The plates were incubated at 37°C for 3 and 5 days for H17 and M45 strains, respectively. After incubation, the spores were scraped up, washed, and resuspended in fresh minimal salt solution containing 1 g of $(NH_4)_2SO_4$, 10 g of KH_2PO_4, 0.1 g of $MgSO_4{\cdot}7H_2O$, and 0.5 g of sodium citrate. Thereafter, they were treated with lysozyme (2 mg/ml) and 1% sodium dodecyl sulfate (SDS) subsequently for 30 min each. The detergent was removed with subsequent washings (minimum of five washings) with sterile distilled water. Spores were suspended in sterile distilled water for storage at 4°C.

rec assay procedure

The *B. subtilis rec* assay was performed according to the liquid method of Kada using the strains *B. subtilis* H17 (rec^+) and *B. subtilis* M45 (rec^-) (Kada *et al.*, 1980). Compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 2 mg/ml. Initially, 0.1-ml portions of the nutrient broth were poured into each well of a plastic 96-well microplate. Next, 0.1 ml of the solution with compounds was added to the first well and mixed. Then 0.1 ml of this mixture was poured into the second one. This procedure was repeated 10 times to prepare serial half-dilutions of the compounds (Sakagami *et al.*, 1988; Sharma and Sobti, 2000). Thereafter, the plates were inoculated with a 40-µl portion of spore solutions of rec^+ and rec^- (1.3×10^8), covered with lids, and incubated overnight at 37°C. The presence or absence of bacterial growth was checked by the measurement of the absorbance at a wavelength of 620 nm in all wells, and minimum inhibitory concentrations (MICs) were compared between rec^+ and rec^- strains. All experi-

ments were performed using three plates. At least three wells were used for each dose and each experiment was repeated three times. Based on the experimental results of the tested compounds, the ratio of 50% lethal dose (Rec_{50}) as given in the equation below was used to assess genotoxicity of the tested compounds (Sakagami *et al.*, 1988).

$$Rec_{50}\ \text{value} = \frac{CR_{50}\ rec^{+}\ (50\%\ \text{lethal dose of}\ B.\ subtilis\ \text{H17})}{CR_{50}\ rec^{-}\ (50\%\ \text{lethal dose of}\ B.\ subtilis\ \text{M45})}$$

To describe the genotoxic potential of a sample, a survival curve was drawn with the logarithmic concentration on the abscissa and survival on the coordinate. The area enclosed between both survival curves of rec^{+} and rec^{-} corresponds to genotoxicity. With the Probit scale transformation, the two curves are converted to two linear functions (Fig. 1). Then the enclosed area between the two lines can be calculated by a simple integration. This integrated area, designated as the S-probit, is a quantitative index for evaluating genotoxic potential (Takigami *et al.*, 2002).

The DNA damaging potentials of the chemicals consist of four ranges of genotoxicity and the criteria of ranges of genotoxicity for S-probit and Rec_{50} values are given in Table 2 (Sakagami *et al.*, 1988)

Results and Discussion

Many clinically important chemotherapeutic drugs induce DNA interstrand crosslinks such as mitomycin C, cyclophosphamide, melphalan, nitrogen and sulfur mustards, cisplatin, and photoactivated psoralens exhibiting alkylating and/or DNA

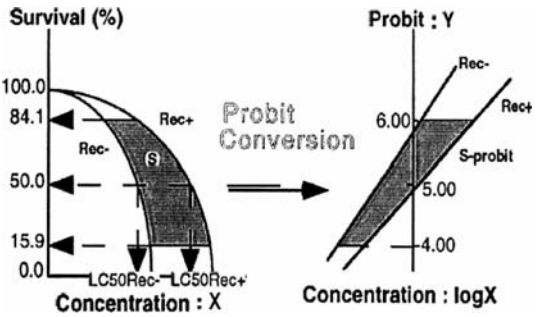


Fig. 1 Survival curves for rec^{+} and rec^{-} tested with a typical genotoxic substance. Conversion to the Probit coordinate is shown on the right, where the S-probit is shown

Table 2 Criteria of ranges of genotoxicity for S-probit and Rec_{50} values

Evaluation of genotoxicity	Rec_{50}	S-probit
Strong genotoxic response (++)	>2	>0.593
Genotoxic response (+)	1.5–1.99	0.200–0.592
Nongenotoxic (–)	0.85–1.49	–0.123–0.199
Reverse effect (*r*)	<0.85	<–0.123

From Takigami *et al.* (2002)

photoreactive activities. Some of these compounds induce DNA monoadducts in addition to interstrand crosslinks. Compared to intrastrand crosslinks, interstrand crosslinks are more cytotoxic, mutagenic, and recombinogenic (Dronkert and Kanaar, 2001; Reddy and Vasquez, 2005).

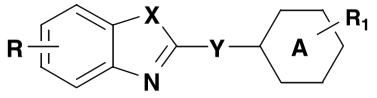
In this study, a congeneric set of newly **(1–6)** and previously **(7–18)** (Sener *et al.*, 1991, 1997; Oren *et al.*, 1997; Temiz *et al.*, 1998; Aki-Sener *et al.*, 2000; Yildiz *et al.*, 2004; Yildiz-Oren *et al.*, 2004) (Formula 1) synthesized isosteric fused heterocyclic compounds of 2,5-disubstituted benzoxazole and benzimidazole derivatives were investigated for their potential *in vitro* genotoxicity by evaluating DNA-damage in the *B. subtilis rec* assay. This assay is a powerful method to recognize wide spectra of DNA damages including intercalation, breakage of DNA molecules, and chemical changes of DNA bases. It can detect mutagens at a much lower concentration than the *E. coli* and *Salmonella* mutation assays, owing to the high permeability of chemicals through the cell membrane of *B. subtilis* (Yamaguchi, 1989). Cisplatin (Sampedro *et al.*, 1991; Khokhar *et al.*, 1993), which is widely used as a cancer chemotherapeutic drug causing DNA damage, was chosen as a standard drug for comparing the activity with the tested compounds.

The rationale for the *B. subtilis rec* assay is based on the relative difference of survival of a DNA repair-recombination proficient strain (rec^+) and its deficient strain (rec^-), which is interpreted as genotoxicity. Recombinationless mutant cells of *B. subtilis* M45 rec^- strains are more sensitive to the cell-killing action of chemical mutagens than the wild-type *B. subtilis* H17 rec^+ strains. Because the sensitivity of the *rec* assay to chemicals having induction activity of DNA damage is higher than that from other screening techniques, this method is a useful tool for the observation of the genotoxic activity (Kada *et al.*, 1980; Konishi and Oki, 1995; Shi *et al.*, 2001). The *B. subtilis rec* assay was applied to various organic and inorganic chemicals including genotoxic and cytotoxic substances, and the criteria used to asses genotoxicity were derived from those results.

All genotoxic activity results of newly and previously synthesized compounds **1–18** together with the positive control reference agent 4-nitroquinoline 1-oxide (4-NQO) and the standard drug cisplatin were given in Table 3. The activity results showed that none of the tested compounds was found more genotoxic than the classical positive mutagen 4-NQO and the standard drug cisplatin. However, among the tested compounds, 6-methyl-2-(o-chloro- phenyl)benzoxazole **(9)**, 5-amino-2-(p-methylbenzyl)benzoxazole **(4)**, 5-(*p*-fluorobenzamido)-2-phenylbenzoxazole **(13)**, and 2-(*p*-methylaminophenyl)benzoxazole **(18)** showed genotoxic activity, with Rec_{50} values of 1.85, 1.74, 1.60, and 1.50 or S-probit values of 0.534, 0.482, 0.460, and 0.357, respectively. Compound **9** exhibited the most potent DNA-damaging property in this set of tested compounds, showing an S-probit value of 0.534 that was in part close to cisplatin. Interestingly, 2-(*p*-bromobenzyl)-5-methylbenzimidazole **(6)** and 2-benzyl-5-(*p*-fluorophenyl-acetamido)benzoxazole **(15)** exhibited a reverse effect that displayed a bacterial growth in the rec^- strains while there was no any bacterial growth in rec^+ strains at the same concentration.

Research conducted with bacterial systems has shown that compounds that cause DNA damage and induce the DNA repair system could also be promising as antitumoral agents in mammals, including humans, as repair-deficient persons

Table 3 Genotoxic activity of the tested compounds **1–18** shown as Rec_{50} and S-Probit values



Compound no.	A	X	Y	R	R_1	MIC (μg/ml) *rec*$^+$	MIC (μg/ml) *rec*$^-$	$Rec_{50} = CR_{50}$ (*rec*$^+$)/ CR_{50} (*rec*$^-$)	S-Probit
1	C_6H_{11}	O	—	5-Cl, 6-NO_2	H	500	500	1.25	0.190
2	C_6H_4	O	CH_2	5-NO_2	4-OC_2H_5	500	500	1.27	0.220
3	C_6H_4	O	CH_2	5-NH_2	4-F	500	500	1.25	0.198
4	C_6H_4	O	CH_2	5-NH_2	4-CH_3	500	500	1.74	0.482
5	C_6H_4	O	—	5-Cl	4-NH_2	2000	2000	1.00	0
6	C_6H_4	NH	CH_2	5-CH_3	4-Br	30	125	0.49	–0.606
7	C_6H_4	O	CH_2O	5-Cl	4-Cl	500	500	1.00	0
8	C_6H_5	O	CH_2O	6-CH_3	H	1000	1000	0.93	0.064
9	C_6H_4	O	—	6-CH_3	2-Cl	125	60	1.85	0.534
10	C_6H_4	O	—	5-CH_3	2-NO_2	500	500	0.96	–0.046
11	C_6H_5	O	—	5-NO_2	H	500	500	1.07	0.056
12	C_6H_{11}	NH	CH_2	5-Cl	H	1000	500	0.93	–0.063
13	C_6H_5	O	—	5-(*p*-flurobenzamido)	H	1000	500	1.60	0.460
14	C_6H_4	O	—	5-(*p*-bromophenylacetamido)	4-C_2H_5	500	500	0.95	–0.020
15	C_6H_5	O	CH_2	5-(*p*-flurophenylacetamido)	H	500	500	0.58	–0.462
16	C_6H_4	O	CH_2	5-Cl	4-Br	1000	1000	1.05	0.047
17	C_6H_4	O	—	5-NH_2	4-Br	1000	1000	1.05	0.045
18	C_6H_4	O	—	H	4-$NHCH_3$	1000	500	1.50	0.357

Table 3 continued

Compound no.	A	X	Y	R	R_1	MIC (μg/ml) rec^+	MIC (μg/ml) rec^-	$Rec_{50} = CR_{50}$ (rec^+)/ CR_{50} (rec^-)	S-Probit
Cisplatin[a]	1000	1000	2.95	0.932					
4-Nitroquinoline 1-oxide (4-NQO)[b]	1000	60	2.21	0.690					

[a] Standard drug

[b] Positive mutagen reference agent

exhibit defects in their ability to repair damaged DNA and thus are prone to developing cancer (Sawicka *et al.*, 1991).

Structure–activity relationships in this set of tested compounds revealed that benzoxazole derivatives were more potent than benzimidazoles for the genotoxic activity. Benzoxazole derivatives possessing most of phenyl and/or rarely benzyl moieties on position 2 in the fused ring system rather than having phenoxymethyl and/or cyclohexyl groups and holding either an amino or *p*-fluorobenzamido groups on position 5 and/or methyl substitution on position 6 enhanced the genotoxic activity. In addition, when attaching a *p*-(methylamino)phenyl group at position 2 of the nonsubstituted, benzoxazole increased the activity. On the other hand, having *p*-fluorophenylacetamido group instead of *p*-fluorobenzamido at the fifth position of 2-benzylbenzoxazole structure showed a reverse effect in genotoxic activity (compare the S-probit values of compounds **15** and **13** in Table 3).

In conclusion, the *B. subtilis rec* assay results show that some of the benzoxazole derivatives have DNA-damaging genotoxic activity that should be considered in further studies.

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