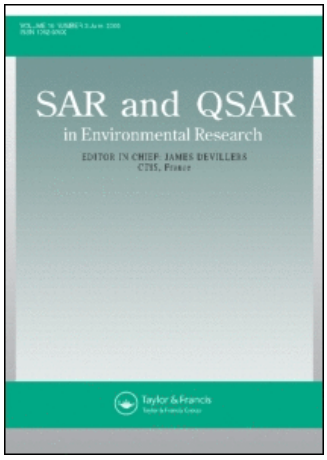

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QSAR of genotoxic active benzazoles§

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Previously synthesized 2,5-disubstituted benzoxazole and benzimidazole derivatives, were tested for their genotoxic activity in the *Bacillus subtilis* rec− assay. The results revealed that 5-methyl-2-(*p*-aminobenzyl)benzoxazole exhibited the highest genotoxic response, which was comparable to 4-nitroquinoline 1-oxide (4-NQO), the reference agent of classical positive mutagen. Among the other tested compounds, four showed a genotoxic activity. A QSAR study revealed that structural parameters $IY_{C_2H_4}$ and IY_{CH_2O}, indicating the bridge elements between the phenyl moiety and the fused ring system at position 2 and the quantum chemical parameter (ΔE), showing the difference between HOMO and LUMO energies, were found significant for enhancing the genotoxic activity in these compounds. In addition, the substituent effects on positions R and R_1 were found important for the activity as well as holding a substituent possessing a maximum length with a minimum width property on position R_1 like alkyl groups. On the other hand, substituting position R with an electron donating group instead of electron withdrawing group increased the genotoxic activity.

Keywords: Benzoxazoles; Benzimidazoles; Genotoxic activity; QSAR

1. Introduction

The benzazoles, which are 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 chemotherapeutic activities. In our previous studies, we reported some derivatives of benzoxazoles, benzimidazoles, and related fused heterocyclic compounds, which exhibited antimicrobial [1, 2], antiviral [3], multi-drug resistance cancer cell activities [4] with

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inhibiting activity on eukaryotic topoisomerase II enzyme in cell-free system [5, 6]. Although benzoxazoles and benzimidazoles are the structural isosters of natural nucleotides and interact easily with the biopolymers, there were no genotoxic studies performed since today showing that these compounds may directly bind to DNA or they may indirectly lead to DNA damage by affecting enzymes involved in DNA replication.

Genotoxic agents showing activity on nucleic acids alter their function by directly binding to DNA or indirectly leading to DNA damage by affecting enzymes involved in DNA replication [7]. It appears primarily in the form of alterations of the phosphate backbone, sugar or base modifications such as alkylation, cross-links or formation of bulky DNA adducts, which are substrates for DNA repair mechanisms. Transient DNA breaks arise in the second step as a consequence of repair and can be considered important markers of genotoxicity [8–10]. The genotoxic chemotherapy treatments include alkylating agents, intercalating agents, enzyme inhibitors [11–18] and these drugs affect both normal and cancerous cells so that genotoxicity of these drugs yields the most serious side effects due to the possibility of inducing secondary malignancies.

In this study, a series of our previously synthesized fused heterocyclic compounds [1, 19–22], 2,5-disubstituted benzoxazole and benzimidazole derivatives, were tested for their genotoxic activity by using the *Bacillus subtilis* rec-assay and QSARs were derived from multivariable regression analysis (MRA). The *B. subtilis* rec-assay is a specially developed test method to detect genotoxicity of a large number of compounds. It has been applied to various organic and inorganic chemicals including genotoxic and cyctotoxic substances and the criteria used to assess genotoxicity were derived from these results [23].

The QSAR analysis was performed using the extra-thermodynamic method, correlating the genotoxic activity with various physicochemical parameters in order to reveal predictions for the lead optimisation in the training set of compounds. The activity contributions for either ring systems or substituent effects at positions 2 and 5 were determined from correlation equations obtained from multivariable regressions analysis (MRA) and the prediction of potent genotoxic derivative was described by the results obtained from the QSAR analysis.

2. Materials and methods

2.1 *Genotoxic study*

Both the bacterial strains *Bacillus subtilis* H17 (arg^-, trp^-, $recE^+$) and *Bacillus subtilis* M45 (arg^-, trp^-, $recE^-$) were obtained from the 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 [24]. The induction of DNA damage and the efficacy of mutation induction were correlated by the screening of positive mutagen agent, 4-nitroquinoline 1-oxide (4-NQO). The strains were checked routinely for ultraviolet-light sensitivity, arginine and tryptophan requirements. They were stored at −80°C.

2.2 *Preparation of spores*

The spores were prepared by spreading overnight broth cultures of strains in different sets of sterile disposable Petri-plates on modified Schaeffer's agar medium [24]. The plates were incubated at 37°C for 3 and 5 days for H17 and M45 strains, respectively. After incubation, the spores were scrapped up, washed and re-suspended in fresh minimal salt solution (containing 1 g $(NH_4)_2SO_4$, 10 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$ and 0.5 g sodium citrate). Thereafter, they were treated with lysozyme ($2\,mg\,mL^{-1}$) and SDS (1%) 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.

2.3 *Strain checking*

The sensitivity of strains was checked frequently by UV experiments. The bacterial strains of *B. subtilis* H17 (rec+) and *B. subtilis* M45 (rec−) were streaked in a single line on Luria agar plate and exposed to germicidal UV lamp (UV Philips lamp emitting at 254 nm VWR Canlab) for different intervals at a distance of 35 cm from the lamp. A section of the plate remained unexposed to UV light. The absence of growth on the unprotected *versus* protected part was taken as evidence for UV sensitivity related to the absence of recombination repair and a functional rec+ gene. The UV sensitivity does not indicate the absence of the rec gene as a mutant lacking excision repair capacity would also demonstrate UV sensitivity. Slight growth on the UV irradiated unprotected section of the plates demonstrates that the wild type rec+ strain is competent for DNA repair and recombination.

2.4 *Rec-assay procedure*

The *Bacillus subtilis* rec-assay was performed according to the liquid method of Kada *et al.* [25] using the strains of *B. subtilis* H17 (rec+) and *B. subtilis* M45 (rec−). Compounds (table 2) were dissolved in dimethylsulfoxide (DMSO) at the concentration of $2\,mg\,mL^{-1}$. Initially, 0.1 mL portion of the nutrient broth was poured into each well of a plastic microtiter plate. Next, 0.1 mL of the solution with compounds was added to the first well and mixed. After that, 0.1 mL of this mixture was poured into the second one. This procedure was repeated 10 times. Thus serial 1/2 dilutions of the compounds were prepared by repetition [24, 26]. All the tested compounds were dissolved in DMSO. Thereafter, the plates were inoculated with 40 μL portion of spore solutions of rec+ and rec− $(1–3 \times 10^8)$, 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 the wavelength of 620 nm in all wells and minimum inhibitory concentrations (MICs) were compared between rec+ and rec− strains. All experiments were performed using three plates. Based upon the experimental results of the tested compounds, the ratio of 50% lethal dose (Rec_{50}) as given in equation below was used to assess genotoxicity of the tested compounds [26].

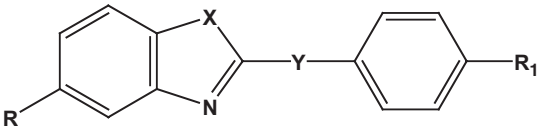
$$Rec_{50}\ \text{value} = \frac{CR_{50}\ \text{rec}+\ (50\%\ \text{lethal dose of } B.\ subtilis\ \text{H17})}{\text{CR50 rec}-\ (50\%\ \text{lethal dose of } B.\ subtilis\ \text{M45})}$$

Evaluation of genotoxicity was made from Rec_{50} values (table 1) [27].

Table 1. Ranges of genotoxicity from Rec_{50} values.

Evaluation of genotoxicity	*Rec_{50} values*
Strong genotoxic response (++)	< 2
Genotoxic response (+)	1.5 to 1.99
Nongenotoxic (–)	0.85 to 1.49

Table 2. Compounds and their genotoxic activities as Rec_{50} values with the MIC values against the rec+ and rec− strains.



Comp. No	*X*	*Y*	*R*	*R_1*	*MIC ($\mu g\,mL^{-1}$) Rec+*	*IC ($\mu g\,mL^{-1}$) Rec−*	*Rec_{50} CR_{50} (Rec+)/ CR_{50}(Rec−)*	*Gen effect*
1	O	–	NH_2	H	1000	1000	1.07	
2	O	–	NH_2	F	4000	4000	0.94	
3	O	–	Cl	NH_2	2000	2000	1	
4	O	–	NO_2	H	500	500	1.07	
5	O	CH_2	H	NO_2	1000	2000	0.59	
6	O	CH_2	NO_2	NO_2	2000	2000	0.9	
7	O	CH_2	H	Cl	1000	1000	1.5	+
8	O	CH_2	CH_3	NH_2	1000	500	2.22	++
9	O	CH_2	NH_2	Cl	500	250	1.8	+
10	NH	C_2H_4	CH_3	H	250	500	0.78	
11	NH	C_2H_4	Cl	H	125	125	1.086	
12	NH	CH_2O	H	H	1000	1000	1.61	+
13	NH	CH_2O	Cl	H	500	500	1.3	
14	NH	CH_2O	Cl	Cl	1000	1000	0.88	
15	NH	CH_2O	NO_2	H	8000	8000	0.724	
16	O	CH_2O	Cl	Cl	500	500	1	
17	O	–	H	$NHCH_3$	1000	500	1.5	+
18	NH	CH_2	CH_3	CH_3	8000	8000	0.93	
19	O	CH_2	NH_2	CH_3	500	500	1.74	+
20	O	CH_2	NH_2	F	500	500	1.25	
21	O	CH_2	Cl	OCH_3	1000	1000	1.4	
*4-Nitroquinoline-1-oxide (4-NQO)					1000	60	2.21	++

*Positive mutagen agent.

3. QSAR analysis

The genotoxic activity results obtained from the *Bacillus subtilis* rec-assay shown as Rec_{50} values and the ranges of genotoxicity of the tested compounds are given in table 2 with the MIC values of the compounds against the rec+ and rec− strains. The potency was defined as $\log 1/C$ where C was the molar Rec_{50} values of the compounds. A training set including compounds 1–16 and a test set consisting in compounds 17–21 were considered. The variables used as independent descriptors in the QSAR analysis were hydrophobic, electronic, steric, and structural parameters.

The screened physicochemical parameters in this QSAR study are $\log P$, for the hydrophobic effects, σ, F (field effect), R (resonance effect), LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) energies (E_{LUMO}, E_{HOMO}) for the quantum chemical parameters with the difference (ΔE) between E_{HOMO} and E_{LUMO} as the electronic influences and Verloop's STERIMOL descriptors (L, B_1, B_4) for the steric interactions of the substituents R and R_1. Additionally, the structural variable IX expresses the exchange between –O– and –NH– groups in the five-membered ring and is represented as 1 for benzoxazole, and 0 for benzimidazole type compounds. The other Free Wilson type structural indicator variable IY showed a value of 1 for the presence of a bridge group and 0 for the absence of it between the *p*-substituted phenyl moiety and the fused ring system at position 2. Moreover, the bridge elements CH_2, C_2H_4, and/or CH_2O between the phenyl moiety and the fused ring system in position 2 are represented by the Free Wilson type structural dummy parameters as IY_{CH_2}, $IY_{C_2H_4}$, and IY_{CH_2O}, respectively [28, 29]. In order to avoid the singularity problem in these Free Wilson type structural dummy parameters, each variable segment occurred at more than once and at least two different positions in the training set to prevent from linear dependences [30–32].

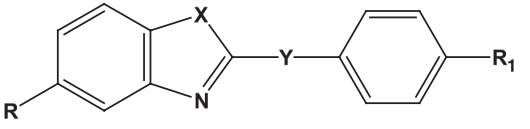
Values of the physicochemical parameters used in this QSAR study were taken from the table of Hansch and Leo [33] except $\log P$, E_{LUMO}, and E_{HOMO}, which were calculated by using the Acclerys's Cerius2 [34] program. The values of the parameters used in the correlation equations (1)–(4) related to the activity among the candidate set of variables in the training set are shown in table 3.

MRA of the QSAR study were run on a PC using the BILIN [35] and MINITAB 13.1 program package [36]. In the equations, the figures in parentheses are the standard errors of the regression coefficients, n is the number of compounds, r^2 is the square of multiple correlation coefficient, F is the significance test and s is the standard error of estimate.

MRA that involves finding the best fit of dependent variable (genotoxic activity) to a combination of independent variables (descriptors) are used by the least squares method. The tabulated $F(4,11,0.95)$ is 3.36 whereas the overall F test values for the obtained equations (1), (2), (3), and (4) were 32.60, 24.72, 22.86, and 16.74, respectively, which are statistically significant at the 5% level of probability [37]. The statistically significant correlation equations (1)–(4) obtained from MRA to describe the QSAR analysis are given in table 4.

In order to judge the validity of the predictive power of the QSAR analysis, the cross-validation method was also applied to the original data set by removing a compound from the data in such a way that each observation (compound) is deleted once and only once. For each reduced data set a model was developed and the response values of the deleted observations were predicted from this model and finally the resulting PRESS (predictive residual sum of squares) and Q^2 (the square of predictive power of coefficient) were calculated for the equations (1)–(4) [38, 39]. The search for the simple correlation coefficients which are given in table 5 also reveals that there is no intercorrelation between the independent variables in any case entered in the correlation equations. The calculated $\log 1/C$ values with residuals of the training set determined from equations (1)–(4) are given in table 6.

Table 3. Training set of compounds and the parameters used in equations (1)–(4).



Comp. No	*R*	*R1*	*X*	*Y*	*Rec50*	*IY$_{CH_2}$*	*IY$_{C_2H_4}$*	*IY$_{CH_2O}$*	*σR*	*LR1*	*B1R1*	*log P*	*E$_{HOMO}$*	*E$_{LUMO}$*	*ΔE*
1	NH_2	H	O	–	1.07	0	0	0	−0.16	2.06	1	2.57	−10.1715	1.7058	−11.8773
2	NH_2	F	O	–	0.94	0	0	0	−0.16	2.65	1.35	2.71	−10.2207	1.5695	−11.7902
3	Cl	NH_2	O	–	1.00	0	0	0	0.37	2.93	1.5	3.08	−10.2416	1.7296	−11.9712
4	NO_2	H	O	–	1.07	0	0	0	0.71	2.06	1	3.30	−11.1589	0.8499	−12.0088
5	H	NO_2	O	CH_2	0.59	1	0	0	0	3.44	1.7	4.01	−11.0787	0.3832	−11.4619
6	NO_2	NO_2	O	CH_2	0.90	1	0	0	0.71	3.44	1.7	3.97	−11.9418	0.1725	−12.1143
7	H	Cl	O	CH_2	1.50	1	0	0	0	3.52	1.8	4.58	−10.8852	2.3466	−13.2318
8	CH_3	NH_2	O	CH_2	2.22	1	0	0	−0.07	2.93	1.5	3.74	−10.4944	2.9642	−13.4586
9	NH_2	Cl	O	CH_2	1.80	1	0	0	−0.16	3.52	1.8	3.79	−10.4422	2.5202	−12.9624
10	CH_3	H	NH	C_2H_4	0.78	0	1	0	−0.07	2.06	1	4.74	−10.5866	3.1473	−13.7339
11	Cl	H	NH	C_2H_4	1.09	0	1	0	0.37	2.06	1	4.8	−10.9363	2.7768	−13.7131
12	H	H	NH	CH_2O	1.61	0	0	1	0	2.06	1	3.54	−10.7460	2.9952	−13.7412
13	Cl	H	NH	CH_2O	1.30	0	0	1	0.37	2.06	1	4.06	−11.1027	2.6042	−13.7069
14	Cl	Cl	NH	CH_2O	0.88	0	0	1	0.37	3.52	1.8	4.58	−11.2115	2.4683	−13.6798
15	NO_2	H	NH	CH_2O	0.72	0	0	1	0.71	2.06	1	3.49	−11.6254	0.9622	−12.5876
16	Cl	Cl	O	CH_2O	1.00	0	0	1	0.37	3.52	1.8	4.75	−11.4658	2.1393	−13.6051

Table 4. Correlation equations used in QSAR analysis.

Equation number	*Equations*	*n*	r^2	*s*	*F*	Q^2	*s-PRESS*
(1)	$\log 1/C = +0.31(\pm0.13)\mathrm{IY}_{\mathrm{C_2H_4}} + 0.33(\pm0.09)\mathrm{IY}_{\mathrm{CH_2O}} + 0.32(\pm0.06)\Delta E + 0.19(\pm0.06)\log P + 5.57(\pm0.69)$	16	0.960	0.055	32.601	0.800	0.088
(2)	$\log 1/C = +0.62(\pm0.16)\mathrm{IY}_{\mathrm{C_2H_4}} + 0.43(\pm0.11)\mathrm{IY}_{\mathrm{CH_2O}} + 0.15(\pm0.06)\mathrm{L}_{\mathrm{R1}} + 0.28(\pm0.07)\Delta E + 5.26(\pm0.76)$	16	0.949	0.062	24.720	0.780	0.092
(3)	$\log 1/C = +0.63(\pm0.17)\mathrm{IY}_{\mathrm{C_2H_4}} + 0.44(\pm0.12)\mathrm{IY}_{\mathrm{CH_2O}} + 0.28(\pm0.07)\Delta E + 0.29(\pm0.12)\mathrm{B1}_{\mathrm{R1}} + 5.35(\pm0.79)$	16	0.945	0.065	22.862	0.770	0.095
(4)	$\log 1/C = -0.25(\pm0.11)\mathrm{IY}_{\mathrm{CH_2}} - 0.21(\pm0.19)\sigma_{\mathrm{R}} - 0.22(\pm0.07)E_{\mathrm{LUMO}} + 0.19(\pm0.08)\log P + 2.17(\pm0.23)$	16	0.927	0.074	16.737	0.733	0.102

Table 5. Correlation matrix of the variables used in equations (1)–(4).

	IY_{CH_2}	$IY_{C_2H_4}$	IY_{CH_2O}	σ_R	log *P*	B_{1R_1}	L_{R_1}	E_{LUMO}	ΔE
IY_{CH_2}	1.000	0.065	0.207	0.060	0.024	0.402	0.425	0.043	0.029
$IY_{C_2H_4}$		1.000	0.065	0.005	0.247	0.162	0.158	0.170	0.159
IY_{CH_2O}			1.000	0.109	0.049	0.010	0.011	0.041	0.249
σ_R				1.000	0.021	0.012	0.009	0.225	0.003
log *P*					1.000	0.089	0.096	0.136	0.481
B_{1R_1}						1.000	0.996	0.024	0.006
L_{R_1}							1.000	0.036	0.008
E_{LUMO}								1.000	0.701
ΔE									1.000

Table 6. Observed and calculated log $1/C$ values with residuals obtained from the equations (1)–(4).

	Equation (1)			*Equation (2)*		*Equation (3)*		*Equation (4)*	
Comp. No	*Observed log 1/C*	*Calculated log 1/C*	*Residuals*	*Calculated log 1/C*	*Residuals*	*Calculated log 1/C*	*Residuals*	*Calculated log 1/C*	*Residuals*
1	2.293	2.293	0.001	2.315	−0.022	2.316	−0.023	2.326	−0.033
2	2.385	2.346	0.039	2.430	−0.045	2.440	−0.055	2.383	0.002
3	2.389	2.358	0.031	2.423	−0.034	2.432	−0.044	2.309	0.079
4	2.351	2.386	−0.035	2.279	0.072	2.280	0.072	2.473	−0.122
5	2.634	2.690	−0.056	2.641	−0.007	2.632	0.003	2.613	0.022
6	2.522	2.477	0.045	2.462	0.060	2.449	0.072	2.504	0.018
7	2.211	2.237	−0.026	2.167	0.043	2.166	0.045	2.293	−0.082
8	2.031	2.010	0.021	2.015	0.016	2.017	0.014	2.011	0.020
9	2.158	2.176	−0.019	2.241	−0.084	2.241	−0.084	2.136	0.021
10	2.481	2.419	0.063	2.425	0.057	2.425	0.057	2.410	0.072
11	2.374	2.436	−0.063	2.430	−0.057	2.430	−0.057	2.411	−0.037
12	2.144	2.213	−0.069	2.235	−0.091	2.233	−0.089	2.198	−0.054
13	2.299	2.320	−0.021	2.245	0.054	2.242	0.057	2.306	−0.007
14	2.523	2.425	0.098	2.476	0.047	2.477	0.046	2.436	0.087
15	2.570	2.568	0.003	2.552	0.018	2.555	0.016	2.485	0.085
16	2.469	2.480	−0.011	2.497	−0.028	2.498	−0.030	2.541	−0.072

4. Discussion

A congener set of previously synthesized isosteric fused heterocyclic compounds, 2,5-disubstituted benzoxazole and benzimidazole derivatives 1–21, were tested for their genotoxic activity using the *B. subtilis* rec-assay. This assay is a powerful and one of the simplest methods 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, due to high permeability of chemicals through the cell membrane of *B. subtilis* [40].

The *B. subtilis* rec-assay test was applied to various organic and inorganic chemicals including genotoxic and cyctotoxic substances and the criteria used to assess genotoxicity were derived from those results [40]. This method has also been used as a tool for pre-screening of anticancer drugs [41–43]. The rationale of the rec-assay test is

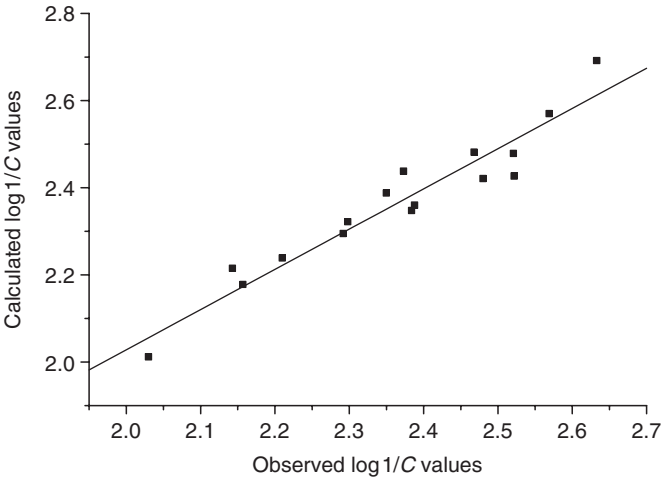


Figure 1. Plot of observed *vs.* calculated $\log 1/C$ values of the training set compounds obtained by using equation (1).

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. Recombination less mutant cells of rec− is more sensitive to the cell-killing action of chemical mutagens than are the wild-type bacterium strain rec+. Since the sensitivity of the rec-assay to chemicals having induction activity of DNA damage is higher than that from other screening techniques, this method may be useful for pre-screening of bioactive compounds in crude drugs, as well as in microorganisms.

The genotoxic activity results regarding the Rec_{50} values of the screened benzoxazole and benzimidazole derivatives 1–21 with the positive control agent 4-nitroquinoline-1-oxide (4-NQO) are given in table 2. The activity results reported in table 2 reveal that 5-methyl-2-(*p*-aminobenzyl)benzoxazole (#8) exhibited the most pronounced strong genotoxic response showing a Rec_{50} value of 2.22, which is comparable with the reference agent of classical positive mutagen 4-NQO. Among the tested compounds, 5-amino-2-(*p*-chlorobenzyl)benzoxazole (#9), 5-amino-2-(*p*-methylbenzyl)benzoxazole (#18), 2-phenoxymethyl-benzimidazole (#12), and 2-(*p*-chlorobenzyl)benzoxazole (#7) show genotoxic activities, having Rec_{50} values of 1.8, 1.74, 1.61, and 1.5, respectively.

Results of QSAR analysis obtained by the linear regression analysis of the training set of compounds 1–16, demonstrate that equations (1)–(4), given in table 4, are statistically significant. Equation (1) represents the best fitted model among the others. As can be deduced from figure 1, the goodness-of-fit of equation (1) is more significant than the other correlation models given in table 4, possessing a high r^2 (96%) and a small s (0.055) with an overall F test value of 32.60 at the significant level of $p < 0.05$. From a statistical point of view, equations (1)–(4) have a sufficient number of DF (degrees of freedom, DF = 11; see table 7) that can be judged significant for overall F and t statistics at the 5% level of probability. As shown in table 7, all the included variables in the equations (1)–(4) possess t values ≥ 2.10 (the tabulated t value for DF = 11; 95% significance level), confirming that the confidence intervals of all individual regression coefficients are justified at the 95% significance level [44, 45].

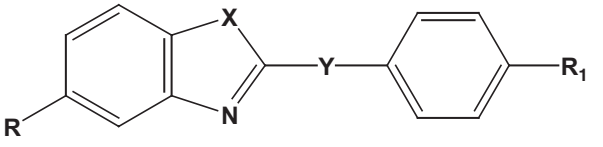
Table 7. Complete analysis of variance table of equations (1)–(4).

	Degrees of freedom	*Sum of squares*	*Mean square*	*t value*	*p level*
Equation (1)					
Total (corrected)	15	0.427			
Regression	4	0.393	0.098		
$IY_{C_2H_4}$	1	0.009	0.009	5.42	0.000
IY_{CH_2O}	1	0.016	0.016	7.90	0.000
ΔE	1	0.246	0.246	11.02	0.000
$\log P$	1	0.122	0.122	6.36	0.000
Residual error	11	0.033	0.003		
Equation (2)					
Total (corrected)	15	0.427			
Regression	4	0.384	0.096		
$IY_{C_2H_4}$	1	0.009	0.009	8.40	0.000
IY_{CH_2O}	1	0.016	0.016	8.43	0.000
L_{R1}	1	0.024	0.024	5.38	0.000
ΔE	1	0.335	0.335	9.29	0.000
Residual error	11	0.043	0.004		
Equation (3)					
Total (corrected)	15	0.427			
Regression	4	0.381	0.095		
$IY_{C_2H_4}$	1	0.009	0.009	8.10	0.000
IY_{CH2O}	1	0.016	0.016	8.16	0.000
ΔE	1	0.246	0.246	9.02	0.000
$B1_{R1}$	1	0.109	0.109	5.13	0.000
Residual error	11	0.046	0.004		
Equation (4)					
Total (corrected)	15	0.427			
Regression	4	0.366	0.092		
IY_{CH_2}	1	0.021	0.021	−4.85	0.001
σ_R	1	0.080	0.080	−2.37	0.037
E_{LUMO}	1	0.106	0.106	−6.83	0.000
$\log P$	1	0.160	0.160	5.40	0.000
Residual error	11	0.060	0.005		

In order to avoid the risk of chance correlation, some circumstances which were pointed out by Kubinyi [30], have been taken into consideration in the study. Cross-validation was applied to the original data set and the resulting PRESS was calculated. The calculated overall PRESS values for equations (1)–(4) are 0.088, 0.092, 0.095, and 0.102, respectively that are found smaller than the SSY (sum of the squares of the response values of the total observations) values of the observed equations (1)–(4), which are 0.427 (see table 7). This proves that the developed models predict better than chance and can be considered statistically significant [39]. The ratio PRESS/SSY for equations (1)–(4), which is the approximate confidence interval for a prediction, are smaller than 0.4 and it also provides proof that the observed models are valid [38, 39].

All the obtained correlation equations (1)–(4) were screened by using a test set (table 8) concerning the compounds 17–21 that are not included in the developed models. Equation (4) was the best fit predicted model among the others showing a r^2 value of 0.691. The observed *versus* predicted $\log 1/C$ values and residuals of the test set molecules obtained by equation (4) are given in table 8.

Table 8. Compounds, parameters, observed and predicted log $1/C$ values with residuals used in equation (4) for the test set.



Comp. No	R	R_1	X	Y	Rec_{50}	$IY_{C_2H_4}$	IY_{CH_2O}	log P	ΔE	Observed log 1/C	Predicted log 1/C	Residuals
17	CH_3	CH_3	NH	CH_2	0.93	0	0	4.82	−13.6495	2.405	2.167	0.238
18	NH_2	CH_3	O	CH_2	1.74	0	0	3.74	−13.3228	2.137	1.998	0.139
19	NH_2	F	O	CH_2	1.25	0	0	3.41	−13.3051	2.287	1.961	0.326
20	Cl	OCH_3	O	CH_2	1.40	0	0	4.32	−13.4628	2.291	2.116	0.175
21	NO_2	OC_2H_5	O	CH_2	1.27	0	0	4.10	−10.6028	2.371	2.361	0.010

In this QSAR analysis, correlation equations (1)–(3) revealed that the indicator parameters $IY_{C_2H_4}$ and IY_{CH_2O} are significant for the genotoxic activity. The compounds possessing an ethylene and/or a phenoxymethylene bridge element between the phenyl group and the fused ring system at position 2 are found to be important and increase the activity. On the other hand, equation (4) displayed that the structural parameter IY_{CH_2}, which illustrated a methylene bridge between the phenyl moiety and the fused ring system at position 2, causes a decrease in the genotoxic activity. The necessity of these bridge elements at position 2 can be described by the conformational influences of the molecule and it shows that the shape of flexibility provides essential role in the activity.

The quantum chemical parameter (ΔE) showing the difference between E_{HOMO} and E_{LUMO} of the tested compounds is found to be important and the molecules have higher energy differences enhancing the genotoxic activity. Furthermore, in equation (4), the quantum chemical parameter showing E_{LUMO} of the tested molecules was found significant playing a reducer role for the genotoxic activity. The smaller E_{LUMO} is the smaller resistance to accept electrons; conversely, the greater E_{HOMO} is the greater electron-donating capability. Compounds that present larger values of E_{HOMO} are more electron donor and the compounds that present smaller values of E_{LUMO} are more electron acceptor. These variables are interpreted as measures of molecular reactivity and stability. As E_{HOMO} increases (relative to other molecules), the molecule is less stable and more reactive. For E_{LUMO}, the situation is the converse [46].

In addition to these features, equations (1) and (4) reveal that the molecular parameter log P is another descriptor considering that the hydrophobic property may cause some increase for the genotoxic activity in these set of compounds. Moreover, equations (2)–(4) demonstrate that substituent effects on the positions R and R_1 are also important for the activity and holding a substituent possessing a maximum length with a minimum width property on position R_1 like as alkyl groups enhancing the activity. On the other hand, equation (4) indicates that the electronic influences of Hammet's aromatic sigma substituent parameter on position R was significant and substituting this position with an electron donating group such as NH_2, $NHCH_3$, CH_3 and OCH_3 instead of electron withdrawing groups such as nitro increases the genotoxic activity.

In conclusion, most of the screened fused heterocyclic compounds exhibited low genotoxic activities except the tested compounds **7**–**9**, **12**, and **18**, which have an effect on nucleic acids and alter their function by directly binding to DNA or indirectly leading to DNA damage by affecting enzymes involved in DNA replication. QSAR analysis revealed that the conformational properties and the molecular orbital energies of the compounds are important for the activity and substituting position R with a group enhancing the electron-donating capability of the fused ring system causes increase in the activity together with having alkyl groups on the position R_1 in these set of compounds.

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