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QSARs OF SOME ANTIBACTERIAL ACTIVE BENZOXAZOLES AGAINST *B. SUBTILIS*

BAZI ANTİBAKTERİYAL ETKİLİ BENZOKSAZOLLERİN *B. SUBTİLİS'* E KARŞI KANTİTATİF YAPI-ETKİ İLİŞKİLERİ

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ABSTRACT

The QSAR analysis of a set of previously synthesized 5-substitutedbenzamido- and 5-substitutedphenylacetamido-2-(p-substituted-phenyl)benzoxazole derivatives, which were tested in vitro for their growth inhibitory activity against Bacillus subtilis, was performed by using the stepwise multiple regression analysis. The resulting QSAR revealed that the substitution at position R_2 is more significant than R and R_1 to improve the antibacterial activity. Hydrophobic and steric effects of substituents at R_2 have an important role for increasing the antibacterial activity compared to other parameters.

Key words: *QSAR, Antibacterial activity, Benzoxazoles*

ÖZET

Bu kantitatif yapı-etki ilişkileri analizinde önceden sentezleri gerçekleştirilmiş ve B. subtilis'e karşı in vitro gelişimlerini inhibe etme aktiviteleri test edilmiş olan 5-sübstitüebenzamido- ve 5-sübstitüefenilasetamido-2-(p-sübstitüefenil)benzoksazol türevlerine basamaklı çoklu regresyon analizi uygulandı. Kantitatif yapı-etki ilişkileri analiz sonuçları, R_2 konumunun R ve R_1'den antibakteriyal aktivite için daha önemli olduğunu ortaya koymuştur. R_2'nin hidrofobik ve sterik etkileri antibakteriyal aktivitenin artması için diğer parametrelerden daha önemlidir.

Anahtar Kelimeler: *kantitatif yapı-etki ilişkileri, antibakteriyal etki, benzoksazoller*

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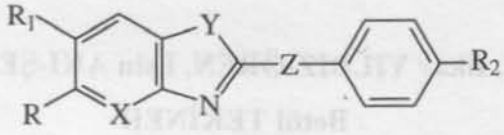
INTRODUCTION

In the last few years, we reported the synthesis and the antimicrobial activity of various 2,5-disubstituted benzoxazoles, benzimidazoles, benzothiazoles and oxazolo[4,5-b]pyridines (Figure 1), against some Gram-positive, Gram-negative bacteria and the yeast *Candida albicans*, providing a wide variety of *in vitro* antimicrobial effects especially indicating significant activity against the enterobacter *Pseudomonas aeruginosa* and the yeast *C. albicans* (1,2).



X ; =CH-, =N-

Y ; -O-, -S-, -NH-

Z ; $-CH_2-$, $-OCH_2-$, $-SCH_2-$, $-C_2H_4-$

R ; -H, -Cl, $-CH_3$, $-NO_2$, $-NH_2$

R_1 ; -H, $-CH_3$, $-NO_2$

R_2; -H, -Cl, -F, -Br, $-CH_3$, $-NO_2$, $-NH_2$, $-C_2H_5$, $-C(CH_3)_3$, $-OCH_3$, $-NHCH_3$, $-NHCOCH_3$, $-N(CH_3)_2$

Figure 1. Previously synthesized 2,5,6-trisubstituted-benzoxazoles, benzimidazoles, benzothiazoles and oxazolo[4,5-b]pyridines.

The determination of the structure-activity relationships of *in vitro* antibacterial and antimycotic activities of the previously synthesized compounds revelead that these related fused heterocyclic systems generally behaved bioisosterically for the screened microorganisms. However, oxazolo[4,5-b]pyridine derivatives showed the best inhibitory potency for the *Klebsiella pneumoniae* and *C. albicans* (3-6).

In order to describe the nature of the interactions at the molecular level, developed QSAR analysis by using the quantum-chemical calculations revelead that the electrophilic superdelocalizability of the nitrogen atom in the oxazolo moiety of the benzoxazole ring and the lowest unoccupied molecular orbital energy levels of the compounds were found in relation with the activity and the fused heterocyclic system was found as the most important part in the molecule for the interactions (5,7).

In the present paper, a set of previously synthesized 2-(p-substituted-phenyl)- 5-substituted-benzamido- and 5-substituted-phenyl-acetamidobenzoxazole derivatives **1-23** were tested for *in vitro* growth inhibitory activity against *B. subtilis* and the QSARs were analyzed by multiple regression analysis (MRA) in order to predict the lead optimization in this set of compounds.

Methodology

The Hansch analysis method has been most widely and effectively used for lead optimization in theoretical drug design. (9,10).

This method can be formulated as given in Eq. 1:

$$\log 1/C = \Sigma a_i I_i + \Sigma b_j X_j + c \qquad \textbf{Eq. 1}$$

where, I_i is the structural indicator parameters and X_j is the physicochemical variables.

In this study, the model is based on the *in vitro* activity of certain 2,5-disubstituted-benzoxazole derivatives **1-23** (Table 1) against *B. subtilis*, where C is the molar concentration of the MIC values of the compounds.

The candidate set of descriptors used in this analysis were π as hydrophobic, σ, F and R as electronic and MW, MR, Es, L, B_1, and B_4 as steric parameters for the substituents R_1 and R_2 (11). Besides these physicochemical variables, structural indicator parameters were also taken into consideration for the substituents Y and R.

The QSAR analysis was performed by using the multiple regression technique and a nonlinear (parabolic) correlation was obtained between antibacterial activity and the lipophilic character of the substituents at position R_2 (12).

On the other side, the predictive power of the performed QSAR model was also determined by using the Cross-Validation Method (13-15).

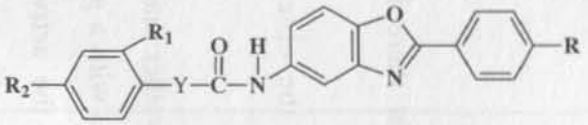
Regression analysis and calculations were run on PC using the BILIN statistical program which was prepared by Hugo Kubinyi (16). In equations, the figures in parenthesis are the standard errors of the regression coefficients. For a given equation, n is the number of compounds, R^2 denotes the square of the multiple correlation coefficients, F is the significance test , s represents the residual standard deviation, Q^2 is the squared cross-validation regression coefficient and s-PRESS showes the standard deviation of cross-validation predictions.

In vitro microbiological activity

The antibacterial activities against the strain *B. subtilis* ATCC 6033 were determined as the minimum inhibitory concentration (MIC) values in vitro by a two-fold serial dilution technique (17,18). The test was performed using the compounds which were dissolved in absolute ethanol (0.4 mg/ml) and further control dilutions in the test medium were furnished at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/ml concentrations. In order to ensure that the solvent per se had no effect on bacterial growth, a control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions used in our experiments and found inactive in culture medium.

For the antibacterial assay, the cultures were obtained in Mueller-Hinton broth (Difco) for all the bacteria after 24 h of incubation at 37 ± 1°C. Testing was carried out in Mueller-Hinton broth at pH 7.4 and the two-fold serial dilution technique was applied. The final inoculum size was 10^5 CFU/ml. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 h at 37 ± 1°C, the last tube with no growth of microorganism was recorded to represent MIC expressed in µg/ml. The potency has been defined as log 1/C in the QSAR analysis where *C* is the molar MIC value of the compounds. MIC and the observed log 1/C values of the tested compounds are listed in Table 1.

Table 1: Compounds and parameters used in **Eq 2** and **3**.



					Physicochemical parameters		Parabolic Model			
Comp. No:	Y	R	R_1	R_2	πR_2	B_4R_2	MIC µg/ml	Obs. Log1/C	Cal. Log1/C	Residuals
1	-	H	H	H	0	1	50	3.798	3.895	-0.097
2	-	H	H	CH_3	0.56	2.04	50	3.817	3.869	-0.052
3	-	H	H	C_2H_5	1.02	2.97	50	3.815	3.690	0.125
4	-	H	H	NO2	-0.28	2.44	200	3.254	3.304	-0.050
5	-	H	H	$C(CH_3)_3$	1.98	2.97	100	3.568	3.561	0.007
6	-	C_2H_5	H	H	0	1	50	3.835	3.895	-0.060
7	-	C_2H_5	H	C_2H_5	1.02	2.97	50	3.869	3.707	0.162
8	-	H	Cl	H	0	1	50	3.843	3.895	-0.052
9	-	H	OCH_3	OCH_3	-0.02	2.87	200	3.272	3.363	-0.091
10	-	H	CH_3	CH_3	0.56	2.04	25	4.136	3.869	0.267
11	CH_2	H	H	H	0	1	50	3.817	3.895	-0.078
12	CH_2	H	H	Br	0.86	1.95	50	3.911	3.969	-0.058
13	CH_2	H	H	Cl	0.71	1.8	50	3.860	3.979	-0.119
14	CH_2	H	H	NO2	-0.28	2.44	200	3.271	3.304	-0.033
15	CH_2	H	H	OC_3H_7	1.05	4.30	200	3.286	3.340	-0.054
16	CH_2	C_2H_5	H	H	0	1	50	3.853	3.895	-0.042
17	CH_2	C_2H_5	H	Br	0.86	1.35	50	3.940	4.135	-0.195
18	CH_2	C_2H_5	H	Cl	0.71	1.8	50	3.893	3.979	-0.086
19	-	H	H	Br	0.86	1.95	50	3.895	3.969	-0.074
20	-	H	H	Cl	0.71	1.8	50	3.843	3.979	-0.136
21	-	F	H	Br	0	1	25	4.120	3.895	0.225
22	OCH_2	$N(CH_3)_2$	H	Cl	0.71	1.8	25	4.180	3.979	0.201
23	SCH_2	$N(CH_3)_2$	H	BH	0	1	25	4.200	3.995	0.205

RESULTS AND DISCUSSION

As a result of QSAR analysis, Eq. 3 was obtained as the best equation for the lead optimization predictions in this set of tested compounds (Table 2). According to the applied stepwise regression technique and the validation test results the performed parabolic correlation equation model given as below.

$$\log 1/C = -0.254\ (\pm 0.16)\ [\pi]^2 R_2 + 0.611\ (\pm 0.25)\ \pi R_2$$

$$- 0.278\ (\pm 0.090)\ B_4 R_2 + 4.173\ (\pm 0.17) \qquad \textbf{Eq. 3}$$

$$\pi\text{-optimum} = 1.20$$

$n = 23$; $R^2 = 0.872$; $s = 0.149$; $F = 20.114$; $p<0.001$

$Q^2 = 0.670$; s-PRESS = 0.175

The correlation coefficients which are given in Table 3 reveal that there is no collinearity between the independent variables used in eq.3.

Compounds and the parameters used in this QSAR analysis together with the observed, calculated and residual values are given in Table 1.

QSAR analysis reveals that the substitution at position R_2 is significant rather than the position R, R_1 and Y for the tested antibacterial activity. Substituting position R_2 with a group which has a hydrophobic character possessing a π value of 1.20 increases the activity . Additionally, it has also found that substituent having a maximum width at this position enhances the activity against *B. subtilis*.

Table 2: Stepwise development of equation 3.

Eq. No.	Equation	n	R^2	s	F	Q^2	s-PRESS
2	Log1/C = - 0.330 (±0.28) $[\pi]^2R_2$+ 0.482 (±0.42) πR_2 + 3.738 (±0.15)	23	0.490	0.259	3.156	-4.127	0.672
3	Log1/C = - 0.254 (±0.16) $[\pi]^2R_2$+ 0.611 (±0.25) πR_2 - 0.278 (±0.090) $B_4 R_2$ + 4.173 (±0.17) **π-optimum = 1.20**	23	0.872	0.149	20.114	0.670	0.175

Table 3: Corelation matrix of variables used in eq. 3.

	πR_2	$B_4 R_2$
πR_2	1.00	0.275
$B_4 R_2$		1.00

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