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 R₂ is more significant than R and R₁ to improve the antibacterial activity. Hydrophobic and steric effects of substituents at R₂ have an important role for increasing the antibacterial activity compared to other parameters.

Key words: QSAR, Antibacterial activity, Benzoxazoles

ÖZET


Anahtar Kelimeler: kantitatif yapılı 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).

![Figure 1](https://example.com/figure1.png)

**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 antifungal activities of the previously synthesized compounds revealed 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 revealed 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:

\[
\log \frac{1}{C} = \sum a_i I_i + \sum b_j X_j + c
\]

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 \(\pi\) as hydrophobic, \(\sigma, F\) and \(R\) as electronic and MW, MR, Es, \(L, B_i\), and \(B_j\) 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 shows 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.

![Chemical structure](image)

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<th>Comp. No.</th>
<th>Y</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>πR₂</th>
<th>B₁R₂</th>
<th>MIC μg/ml</th>
<th>Obs. Log₁₀/C</th>
<th>Cal. Log₁₀/C</th>
<th>Residuals</th>
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<td>4.200</td>
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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.

\[
\log \frac{1}{C} = -0.254 (\pm 0.16) [\pi] R_2 + 0.611 (\pm 0.25) \pi R_2
\]

\[
-0.278 (\pm 0.090) B_\sigma R_2 + 4.173 (\pm 0.17)
\]

\[\pi\text{-optimum} = 1.20\]

\[n = 23; R^2 = 0.872; s = 0.149; F = 20.114; p < 0.001\]

\[Q^2 = 0.670; s\text{-PRESS} = 0.175\]

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 \(\pi\) 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.

<table>
<thead>
<tr>
<th>Eq. No.</th>
<th>Equation</th>
<th>n</th>
<th>R²</th>
<th>s</th>
<th>F</th>
<th>Q²</th>
<th>s-PRESS</th>
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<td>2</td>
<td>Log1/C = - 0.330 (±0.28) $\pi^2R_2 + 0.482 (±0.42) \pi R_2 + 3.738 (±0.15)$</td>
<td>23</td>
<td>0.490</td>
<td>0.259</td>
<td>3.156</td>
<td>-4.127</td>
<td>0.672</td>
</tr>
<tr>
<td>3</td>
<td>Log1/C = - 0.254 (±0.16) $\pi^2R_2 + 0.611 (±0.25) \pi R_2 - 0.278 (±0.090)$ $B_4 R_2 + 4.173 (±0.17)$</td>
<td>23</td>
<td>0.872</td>
<td>0.149</td>
<td>20.114</td>
<td>0.670</td>
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$\pi$-optimum = 1.20

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

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<th></th>
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<th>$B_4$ R$_2$</th>
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<td>$B_4$ R$_2$</td>
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Acknowledgment

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