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Bazı 5-Süstitüe-2-(p-Süstitüebenzil)benzoksazollerin Sentez,  
Antibakteriyal Etki ve Free-Wilson Analizi Kullanılarak Kantitatif  
Yapı-Etki İlişkileri Çalışmaları

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### SUMMARY

In this research some 5-substituted-2-(p-substitutedbenzyl) benzoxazole derivatives were synthesized and their antibacterial activity against *Staphylococcus aureus* was determined using progressive double dilution technique. The compounds were found significantly active (MIC = 6.25-50 µg / mL).

A comparative structure-activity relationships for a series of antimicrobial active 2-benzylbenzoxozoles were investigated by Free-Wilson analysis. The structural parameters were used in the multiple regression analysis.

The results of Free-Wilson analysis suggest that the 5<sup>th</sup> position of the 2-benzylbenzoxazoles has much more significance for the activity than the para position of the benzyl group. The multiple regression analysis also indicate that the 5-CH<sub>3</sub> and p-Cl groups are the most favourable substituents among the others.

**Key Words:** 5-Substituted-2-(p-substitutedbenzyl)benzox azoles, Antibacterial Activity, *Staphylococcus aureus*, Free-Wilson, QSAR.

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## ÖZET

Bu çalışmada bazı 5-süstitüe-2-(p-süstitüebenzil) benzoksazol türevleri sentezlenmiş ve *Staphylococcus aureus*'a karşı antibakteriyal etkileri Tüpte Dilüsyon Yöntemi kullanılarak belirlenmiştir. Bileşikler yeterince etkili bulunmuştur (MİK = 6.25-50 µg / mL).

Bir seri antimikrobiyal etkili 2-benzilbenzoksazol türevlerinin karşılaştırmalı yapı-etki ilişkileri Free-Wilson analizi yardımıyla incelenmiştir. Çoklu regresyon analizinde yapısal parametreler kullanılmıştır.

Free-Wilson analizinin sonucunda 2-benzilbenzoksazollerin 5. konumunun, benzil grubunun para pozisyonuna göre etki için daha önemli olduğu saptanmıştır. Çoklu regresyon analizleri ile 5-CH<sub>3</sub> ve p-Cl gruplarının diğerlerine göre daha uygun olduğu bulunmuştur.

**Anahtar kelimeler:** 5-Sübslitüc-2-(p-süstitüebenzil)benzoksazoller, Antibakteriyal Etki, *Staphylococcus aureus*, Free Wlison, QSAR

## INTRODUCTION

The synthesis and the microbiological activity of various 2-(p-substitutedbenzyl)benzoxazoles having -H, -NO<sub>2</sub>, -Cl groups at the 5<sup>th</sup> position have been synthesized by our research team before (1-3) and also we have stated the Free-Wilson analysis results of these derivatives using their antifungal activity against *Candida albicans* (4).

In this study, we decide to synthesize some more 2-benzylbenzoxazoles carrying -CH<sub>3</sub> group at the 5<sup>th</sup> position and determine the antibacterial activity against *Staphylococcus aureus*, in order to interpret the nature and the effects of the substituents using the Free-Wilson analysis.

The basic asumption of Free-Wilson analysis is that within a homologous series of drugs, individual segments of molecules make additive and constant contributions to biological activity. If such contributions are known, biological activity can be estimated by simple addition for all the compounds obtainable by any new combination of segments involved (5-7).

In this study, 2-benzylbenzoxazole has been chosen as a constant molecule. A two-dimensional set of congeners has been obtained by substituting this molecule at the 5<sup>th</sup> and the para position of the benzyl group at the 2<sup>nd</sup> position. Using these structural parameters

as molecular descriptors in Free-Wilson analysis, the most favourable substituents have been searched. It has also been tried to find out the most significant position for antibacterial activity against *S. aureus*.

## EXPERIMENTAL

### Chemistry

Kieselgel HF<sub>254</sub> chromatoplates (0.3 mm) were used for TLC and the solvent system was only chloroform. Melting points were determined on a Mettler FP-51 apparatus and were uncorrected. IR spectra were recorded with Pye Unicam SP-1025 with KBr discs. <sup>1</sup>H NMR spectra were obtained with a Perkin-Elmer R-32 spectrometer in trifluoroacetic acid and tetramethylsilane as internal standard. UV maxima were measured on a Pye Unicam SP-1700 spectrophotometer in methanol at 10<sup>-3</sup> M concentration. Elemental analysis was carried out with a Perkin-Elmer model 240-C apparatus. The results of elemental analysis (C, H, N) were within  $\pm 0.4\%$  of the calculated amounts. The starting compounds and the solvents were commercially available products.

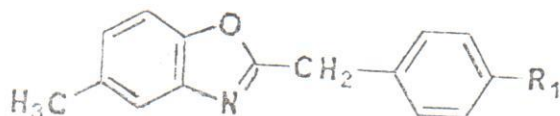
*General procedure:* 5-methyl-2-(p-substitutedbenzyl)benzoxazoles: A mixture of 2-hydroxy-5-methylanilin (0.01 mol) and appropriate phenylacetic acids (0.02 mol) was heated in PPA (12 g) with stirring for 1.5–2.5 h. At the end of the reaction period, the residue was poured into ice-water and neutralized with excess of 10% NaOH solution. After extracted with benzene, the benzene solution was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was boiled with 200 mg charcoal in ether and filtered. The filtrate was left to crystallize by addition of petroleum ether. Chemical and physical data of the compounds are reported in Table 1.

### Microbiology

The activity of the compounds against *Staphylococcus aureus* ATSS 6538 were tested in Mueller Hinton broth. The gradual double dilution technique was applied. After inoculation with 0.2 mL of culture from the Nutrient broth, the seeded broths were incubated



Table 1. Physical and Spectral data of 5-methyl-2-(p-substitutedbenzyl)benzoxazoles.



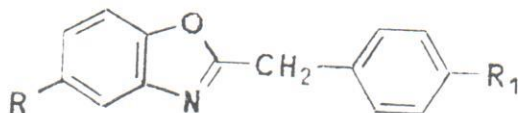
Comp. No:	R <sub>1</sub> <sup>a</sup>	Mp (°C)	Yield (%)	Reaction temp (°C)	UV λ <sub>max</sub>	logε	NMR δ ppm	IR cm <sup>-1</sup>
1	NO <sub>2</sub> <sup>b</sup>	91	50	140–145	211 236 278 285	4.26 4.13 4.21 4.16	2.50 (3H, s), 4.40 (2H, s) 7.00–7.70 (5H, m). 8.10–8.40 (2H, d)	3100,2920, 1620,1520, 1530,1350, 1260
2	NH <sub>2</sub> <sup>b</sup>	64	21	190–195	210 239 278 286	4.25 4.32 3.97 3.93	2.50 (3H, s), 4.20 (2H, s) 3.60 (2H, s), 6.55–6.85 (2H, d). 7.00–7.50 (5H, m)	3420,3250, 3100,2920, 1625,1520, 1480,1260
3	Cl <sup>b</sup>	48	42	130–135	211 278 286	4.23 3.71 3.64	2.45 (3H, s), 4.20 (2H, s) 7.00–7.60 (7H, m)	3100,2950, 1620,1480, 1250
4	Br <sup>b</sup>	52	44	120–130	231 278 286	4.23 3.72 3.65	2.50 (3H, s), 4.20 (2H, s) 7.00–7.80 (7H, m)	3090,2960, 1630,1570, 1485,1260
5	H <sup>b</sup>	44	36	120–125	210 235 286	4.23 4.09 3.67	2.50 (3H, s), 4.30 (2H, s) 7.00–7.60 (BH, m)	3100,2950, 1620,1570, 1480,1250

a– The spectral data of the compounds are obtained in this research

b– Crystallization solvent: ether–petroleum ether.

at 37° C for 24 hours. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 hours, the last tube with no growth of the microorganism was taken to represent the Minimum Inhibitory Concentration (MIC, expressed in µg/mL).

The activities of the compounds were tested in absolute alcohol. For that reason, the activity of ethyl alcohol against *S. aureus* has been tested in the same dilutions and found inactive. The antibacterial activities of the compounds were given in Table 2.

Table 2. The antimicrobial activity 5-substituted-2-(p-substitutedbenzyl)-benzoxazole derivatives against *S. aureus* (MIC in  $\mu\text{g}/\text{mL}$ ).

Comp. No:	R	R <sub>1</sub>	MIC ( $\mu\text{g}/\text{mL}$ )
1	H	H	50
2	H	Br	50
3	H	Cl	50
4	H	NO <sub>2</sub>	50
5	Cl	H	50
6	Cl	Br	50
7	Cl	NO <sub>2</sub>	50
8	Cl	Cl	50
9	NO <sub>2</sub>	H	50
10	NO <sub>2</sub>	Br	50
11	NO <sub>2</sub>	Cl	50
12	NO <sub>2</sub>	NO <sub>2</sub>	50
13	CH <sub>3</sub>	H	12.5
14	CH <sub>3</sub>	Br	12.5
15	CH <sub>3</sub>	Cl	6.25
16	CH <sub>3</sub>	NO <sub>2</sub>	25

### Free - Wilson Analysis

Regression analysis equation of QSAR study has been performed by using IBM-computer working with Stagraft 2.6 Statistic Package.

The Free-Wilson approach is an application of multiple regression analysis of QSAR methodology. This model assumes that for

$$\log 1 / C = \sum a_i x_i + \mu \quad (3)$$

$a$  = contribution of the  $i^{\text{th}}$  substituent

$\mu$  = the overall average activity calculated for the unsubstituted compound.

Table 3. Structure matrix of the compounds derived from Free-Wilson Model.

[illegible]

linear equations in the analysis are performed (Eqs. 4) At the next step, symmetry equations have been observed from Table 4.

Table 4. Correlation matrix derived from symmetry equations.

Comp. No:	R ( $a_1$ )			R <sub>1</sub> ( $a_2$ )			BA
	Cl ( $a_{12}$ )	NO <sub>2</sub> ( $a_{13}$ )	CH <sub>3</sub> ( $a_{14}$ )	Br ( $a_{22}$ )	Cl ( $a_{23}$ )	NO <sub>2</sub> ( $a_{24}$ )	log 1 / C
1	-1	-1	-1	-1	-1	-1	3.6212
2	-1	-1	-1	1	0	0	3.7597
3	-1	-1	-1	0	1	0	3.6875
4	-1	-1	-1	0	0	1	3.7059
5	1	0	0	-1	-1	-1	3.6875
6	1	0	0	1	0	0	3.8089
7	1	0	0	0	0	1	3.7612
8	1	0	0	0	1	0	3.7451
9	0	1	0	-1	-1	-1	3.7059
10	0	1	0	1	0	0	3.8096
11	0	1	0	0	1	0	3.7612
12	0	1	0	0	0	1	3.7767
13	0	0	1	-1	-1	-1	4.2514
14	0	0	1	1	0	0	4.3824
15	0	0	1	0	1	0	4.6149
16	0	0	1	0	0	0	4.0302
Σ	0	0	0	0	0	0	

$$1) \log 1 / C = a_{11} + a_{21} + \mu = 3.6212$$

$$2) \log 1 / C = a_{11} + a_{22} + \mu = 3.6794$$

$$3) \log 1 / C = a_{11} + a_{23} + \mu = 3.7597$$

$$4) \log 1 / C = a_{11} + a_{24} + \mu = 3.6875$$

$$5) \log 1 / C = a_{12} + a_{21} + \mu = 3.7059$$

$$6) \log 1 / C = a_{12} + a_{22} + \mu = 3.6875$$

$$7) \log 1 / C = a_{12} + a_{24} + \mu = 3.7380$$

$$8) \log 1 / C = a_{12} + a_{23} + \mu = 3.8089$$

(4)



- 9)  $\log 1 / C = a_{13} + a_{21} + \mu = 3.7612$
- 10)  $\log 1 / C = a_{13} + a_{22} + \mu = 3.7451$
- 11)  $\log 1 / C = a_{13} + a_{23} + \mu = 3.7059$
- 12)  $\log 1 / C = a_{13} + a_{24} + \mu = 3.7543$
- 13)  $\log 1 / C = a_{14} + a_{21} + \mu = 3.8096$
- 14)  $\log 1 / C = a_{14} + a_{22} + \mu = 3.7612$
- 15)  $\log 1 / C = a_{14} + a_{23} + \mu = 3.7767$
- 16)  $\log 1 / C = a_{14} + a_{24} + \mu = 4.2514$

The Symmetry equations for our sample have been:

$$4a_{11} + 4a_{12} + 4a_{13} + 4a_{14} = 0 \quad (5)$$

$$4a_{21} + 4a_{22} + 4a_{23} + 4a_{24} = 0 \quad (6)$$

$a_{11}$  and  $a_{21}$  have been selected at each position as a dependent variable from the equations 1 and 2.

$$a_{11} = -a_{12} - a_{13} - a_{14} \quad (7)$$

$$a_{21} = -a_{22} - a_{23} - a_{24} \quad (8)$$

Equations set 4, 7 and 8 are combined as substitutes for  $a_{21}$  and  $a_{11}$  in the equation set 4 with the expressions obtained from the equations 7, 8. Descriptor values in the multiple regression analysis were obtained from the correlation matrix derived from symmetry equations (Table 4) and the  $\log 1 / C$  has been used as dependent variable.

## RESULTS and DISCUSSION

In this study, 5-methyl-2-(p-substitutedbenzyl)benzoxazole derivatives were synthesized as novel products by heating 2-hydroxy-5-methylaniline with the p-substitutedphenylacetic acids, in the presence of polyphosphoric acid at different temperatures (Table 1) (8-13).

The in vitro microbiological activity of these compounds was determined against *S. aureus*. The Minimum Inhibitory Concentrations (MIC) were determined using the method of two-fold double dilution technique (14, 15). The compounds were found significantly active (MIC = 6.25-25  $\mu\text{g/mL}$ ) (Table 2).

For QSAR studies using the Free-Wilson Approach, antibacterial active 5-H, -Cl, -NO<sub>2</sub>, -CH<sub>3</sub> substituted 2-(p-substitutedbenzyl) benzoxazoles against *S. aureus* were chosen. As a result of these studies, it is found that the differences between calculated and observed log 1 / C values are very small having good R<sup>2</sup> and standart deviations (Table 5-6). Table 5 also shows the activity contributions of the substituents at both positions.  $\mu$  value shows the value of log 1 / C for the unsubstituted compound.

Table 5. Activity contributions and statistical data.

R		R <sub>1</sub>	
a <sub>11</sub> (H)	-0.2587	a <sub>21</sub> (H)	-0.0653
a <sub>12</sub> (Cl)	-0.1088	a <sub>22</sub> (Br)	0.0583
a <sub>13</sub> (NO <sub>2</sub> )	-0.0953	a <sub>23</sub> (Cl)	0.0703
a <sub>14</sub> (CH <sub>3</sub> )	0.4628	a <sub>24</sub> (NO <sub>2</sub> )	-0.0633
$\mu = 3.8568$			
n = 16, R <sub>2</sub> = 0.8430, s = 0.1065, F = 17.1048			
(confidence level % 99)			

The regression equation obtained from the Free-Wilson analysis is:

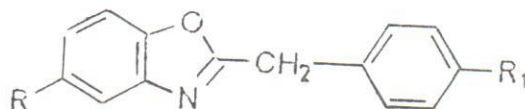
$$\text{BA} = 3.8569 - 0.1088 (\pm 0.106) a_{12} - 0.0953 (\pm 0.106) a_{13} + 0.4628 (\pm 0.106) a_{14} + 0.0583 (\pm 0.106) a_{22} + 0.0703 (\pm 0.106) a_{23} - 0.0633 (\pm 0.106) a_{24}$$

The range of activity contribution values for the substitution site provides information about the sensitivity of biological activity to the variation of substituents in that position (7). For our example the equations are:

$$R = a_{14} - a_{11} = 0.463 - (-0.2346) = 0.6976 \quad (1)$$

$$R_1 = a_{23} - a_{21} = 0.0703 - (0.0653) = 0.1356 \quad (2)$$

The most favourable substituents in the series are methyl as R and chloro as R<sub>1</sub>. It appears that the 5<sup>th</sup> position of 2-benzylbenzoxazole has much more significance than the para position of the benzyl group at the 2<sup>nd</sup> position. It can also be concluded that electron attracting groups at the 5<sup>th</sup> position reduce the activity where as electron releasing group increases.

Table 6. The antibacterial activity of the compounds (MIC:  $\mu\text{g}/\text{mL}$ ), observed and calculated values of  $\log 1/C$ .

Comp. No:	R	R <sub>1</sub>	MIC ( $\mu\text{g}/\text{mL}$ )	$\log 1/C$ Observed	$\log 1/C$ Calculated	Residual
1	H	H	50	3.6212	3.6254	-0.0042
2	H	Br	50	3.6794	3.6907	-0.0113
3	H	Cl	50	3.7597	3.7490	0.0106
4	H	NO <sub>2</sub>	50	3.6875	3.7610	-0.0735
5	Cl	H	50	3.7059	3.6274	0.0784
6	Cl	Br	50	3.6875	3.6828	0.0046
7	Cl	NO <sub>2</sub>	50	3.7380	3.7481	-0.0101
8	Cl	Cl	50	3.8089	3.8064	0.0024
9	NO <sub>2</sub>	H	50	3.7612	3.6848	0.0763
10	NO <sub>2</sub>	Br	50	3.7451	3.8184	-0.0733
11	NO <sub>2</sub>	Cl	50	3.7059	3.6962	0.0096
12	NO <sub>2</sub>	NO <sub>2</sub>	50	3.7543	3.7615	-0.0072
13	CH <sub>3</sub>	H	12.5	3.8096	3.8198	-0.0102
14	CH <sub>3</sub>	Br	12.5	3.7612	3.8318	-0.0706
15	CH <sub>3</sub>	Cl	6.25	3.7767	3.6982	0.0784
16	CH <sub>3</sub>	NO <sub>2</sub>	25	4.2514	4.2543	-0.0029

## ACKNOWLEDGMENT

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