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Synthesis, Antibacterial Activity and QSAR's of some 5-Substituted-2-(p-Substituted benzyI)benzoxazoles using the Free-Wilson Analysis

Bazı 5-Sübstitüe-2-(p-Sübstitüebenzil)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^{th} position of the 2-benzylbenzoxazoles has much more significange for the activity than the para position of the benzyl group. The multiple regression analysis also indicate that the 5-CH_3 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-sübstitüe-2-(p-sübstitüebenzil) benzoksazol türevleri sentezlenmiş ve *Staphylococcus aureus'a* karşı antibakteriyal etkileri Tüpte Dilüsyon Yöntemi kullanılarak belirlenmiştir. Bileşikler yeterince etkili bulunmuştur (MİK = 6.25-50 u*g/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_3 ve p-Cl gruplarının diğerlerine göre daha uygun olduğu bulunmuştur.

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

INTRODUCTION

The synthesis and the microbiological activity of various 2-(p-substitutedbenzyl)benzoxazoles having -H, -NO_2, -Cl groups at the 5th 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_3 group at the 5th position and determine the antibacterial activity against *Staphylococcus aureus,* in order to interprete 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 5th and the para position of the benzyl group at the 2nd position. Using these structural parameters

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

EXPERIMENTAL

Chemistry

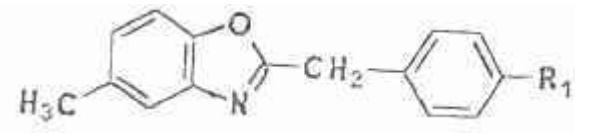
Kieselgel HF254 chromatoplates (0.3 mm) were used for TLC and the solvent system was only chloroform. Melting points were determined on a Mettler FP-51 apparatus and were uncorrected. IR spectra were recorded with Pye Unicam SP-1025 with KBr discs. ^{1}H NMR spectra were obtained with a Perkin-Elmer R-32 spectrometer in trifluoroacetic acid and tetramethylsilane as internal standart. UV maxima were measured on a Pye Unicam SP-1700 spectrophotometer in methanol at 10^{-3} M concentration. Elemental analysis were carried out with a Perkin-Elmer model 240-C apparatus. The results of elemental analysis (C, H, N) were within ± 0.4 % of the calculated amounts. The starting compounds and the solvents were commercially available products.

General procedure: 5-methyI-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.



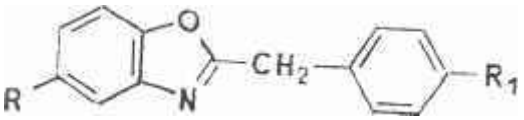
Comp. No:	R_1[a]	Mp (°C)	Yield (%)	Reaction temp (°C)	UV Xmax	logs	NMR δ ppm	IR cm^{-1}
1	NO_2[b]	91	50	140-145	211 236 278 285	4.26 4.13 4.21 4.16	2.50(3H, s), 4.40 (2H, s) 7.00-7.70 (5H, m). 8.10-8.40 (2H,d)	3100,2920, 1620,1520, 1530,1350, 1260
2	NH_2[b]	64	21	190-195	210 239 278 286	4.25 4.32 3.97 3.93	2.50 (3H, s), 4.20 (2H, s) 3.60 (2¾ s), 6.55-6.85(2¾ d). 7.00-7.50(5¾ m)	3420,3250, 3100,2920, 1625,1520, 1480,1260
3	Cl[b]	48	42	130-135	211 278 286	4.23 3.71 3.64	2.45 (3¾ s), 4.20 (2¾ s) 7.00.7.60 (7¾ m)	3100,2950, 1620,1480, 1250
4	Br[b]	52	44	120-130	231 278 286	4.23 3.72 3.65	2.50(3¾ s), 4.20(2¾ s) 7.00-7.80 (7¾ m)	3090,2960, 1630,1570, 1485,1260
5	H[b]	44	36	120-125	210 235 286	4.23 4.09 3.67	2.50 (3¾ s), 4.30(2¾ 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 ug / mL).



Comp. No:	R	R_1	MIC ug/mL)
1	H	H	50
2	H	Br	50
3	H	C1	50
4	H	NO_2	50
5	C1	H	50
6	C1	Br	50
7	C1	NO_2	50
8	CI	CI	50
9	NO_2	H	50
10	NO_2	Br	50
11	NO_2	C1	50
12	NO_2	NO_2	50
13	CH_3	H	12.5
14	CH_3	Br	12.5
15	CH_3	C1	6.25
16	CH_3	NO_2	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

a set of congeners, the biological activity is an additive property of substituents. Quantitatively, Free-Wilson additivity model is given by equation below (6):

$$\log 1 / C == \Sigma_{a_i X_i} + \mu \quad (3)$$

C = Molar concentration of the MIC values

a = contribution of the i^{th} substituent

χ = a value of 1 otherwise a value of O.

μ = the overall average activity calculated for the unsubstituted compound.

At the first step, the structure matrix has been drawn up by listing the structural parameters χ and log 1 / C values of the compounds which were given in Table 3. According to the structural matrix

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

Comp. No:	R(a.$_1$)				R_1 (a_2)				BA MIC ug/mL)
	H (a_{11})	CI (½)	$N0_2$ (a_{13})	Me (a»)	H (a_{21})	Br (a_{22})	CI (a_{23})	$N0_2$ (a_{24})	
1	1	0	0	0	1	0	0	0	50
2	1	0	0	0	0	1	0	0	50
3	1	0	0	0	0	0	1	0	50
4	1	0	0	0	0	0	0	1	50
5	0	1	0	0	1	0	0	0	50
6	0	1	0	0	0	1	0	0	50
7	0	1	0	0	0	0	0	1	50
8	0	1	0	0	0	0	1	0	50
9	0	0	1	0	1	0	0	0	50
10	0	0	1	0	0	1	0	0	50
11	0	0	1	0	0	0	1	0	50
12	0	0	1	0	0	0	0	1	50
13	0	0	0	1	1	0	0	0	12.5
14	0	0	0	1	0	1	0	0	12.5
15	0	0	0	1	0	0	1	0	6.25
16	0	0	0	1	0	0	0	1	25
Sums	4	4	4	4	4	4	**4**	4	

lineer 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.

	R(a_1)			R_1 (a_2)			BA
Comp. No:	Cl (a_{12})	NO_2 (a_{13})	CH_3 (a_{14})	Br (a_{21})	Cl (a_{23})	NO_2 (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	

$$
\begin{aligned}
&1)\ \log\ 1/C = a_{11} + a_{21} + \mu = 3.6212\\
&2)\ \log\ 1/C = a_{11} + a_{22} + \mu = 3.6794\\
&3)\ \log\ 1/C = a_{11} + a_{23} + \mu = 3.7597\\
&4)\ \log\ 1/C = a_{11} + a_{24} + \mu = 3.6875\\
&5)\ \log\ 1/C = a_{12} + a_{21} + \mu = 3.7059\\
&6)\ \log\ 1/C = a_{12} + a_{22} + \mu = 3.6875\\
&7)\ \log\ 1/C = a_{12} + a_{24} + \mu = 3.7380\\
&8)\ \log\ 1/C = a_{12} + a_{23} + \mu = 3.8089
\end{aligned}
\qquad \textbf{(4)}
$$

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) $\log 1/C = \mathbf{a_{14} + a_{22} + \mu =} 3.7612$

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 precence 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 μg/mL) (Table 2).

For QSAR studies using the Free-Wilson Approach, antibacterial active 5-H, -Cl, $-NO_2$, $-CH_3$ 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^2 and standart deviations (Table 5-6). Table 5 also shows the activity contributions of the substitutents at both positions. μ value shows the value of log 1 / C for the unsubstituted compound.

Table 5. Activity contributions and statistical data.

R		R_1	
a_{11}(H)	-0.2587	a_{21} (H)	-0.0653
a_{12}(Cl)	-0.1088	a_{22}(Br)	0.0583
a_{13}(NO_2)	-0.0953	a_{23}(Cl)	0.0703
a_{14}(CH_3)	0.4628	a_{24}(NO_2)	-0.0633
μ = 3.8568			
n= 16, R_2 = 0.8430,		s = 0.106,.F = 17.1048	
(confidence level % 99,			

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

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

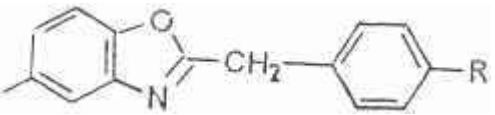
The range of activity contribution values for the substitution site provides information about the sensitivity of biological activity to the variation of substituents in that position (7). For our example the equations are:

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

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

The most favourable substituents in the series are methyl as R and chloro as R_1. It appears that the 5th position of 2-benzylbenzoxazole has much more significance than the para position of the benzyl group at the 2nd position. It can also be concluded that electron atracting groups at the 5th position reduce the activity where as electron releasing group increases.

Table 6. The antibacterial activity of the compounds (MIC: μg/mL), observed and calculated values of log 1 / C.



Comp. No:	R	R,	MIC ug/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	CI	50	3.7597	3.7490	0.0106
4	H	NO_2	50	3.6875	3.7610	-0.0735
5	CI	H	50	3.7059	3.6274	0.0784
6	C1	Br	50	3.6875	3.6828	0.0046
7	CI	NO_2	50	3.7380	3.7481	-0.0101
8	CI	CI	50	3.8089	3.8064	0.0024
9	NO,	H	50	3.7612	3.6848	0.0763
10	NO_2	Br	50	3.7451	3.8184	-0.0733
11	NO,	CI	50	3.7059	3.6962	0.0096
12	NO_2	NO_2	50	3.7543	3.7615	-0.0072
13	CH_3	H	12.5	3.8096	3.8198	-0.0102
14	CH_3	Br	12.5	3.7612	3.8318	-0.0706
15	CH_3	CI	6.25	3.7767	3.6982	0.0784
16	CH_3	NO_2	25	4.2514	4.2543	-0.0029

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REFERENCE

1. Noyanalpan, N., Şener, E., "2-(p-sübstitüebenzil) benzoksazol türevlerinin sentez, yapı aydınlatması ve antihistaminik etkileri", *FABAD, Farm. Bil. Der.* 10, 275-286 (1985).

2. Noyanalpan, N., Şener, E., "5-Kloro-2-(p-sübstitüebenzil) benzoksazol türevlerinin sentez, yapı aydınlatması ve antihistaminik etkileri", *FABAD, Farm. Bil. Der.* 11, 22-30, (1986).

3. Noyanalpan, N., Şener, E., "5-Nitro-2-(p-sübstitüebenzil) benzoksazol türevlerinin sentez, yapı aydınlatması ve antihistaminik etkileri", *FABAD, Farm. Bil. Der.* 11, 111-119, (1986).

4. Şener, E., Yalçın, I., Akın, A., Noyanalpan, N., "Antifungal activity of 2-Benzylbenzoxazole derivatives and QSARs by Free-Wilson Analysis", *Gazi Ecz. Fak. Der.* 4 (1), 1-9, (1987).

5. Free, S.M. and Wilson, J.M., A mathematical contribution to Structure-Activity Studies" *J. Med. Chem.,* 7, 395-399 (1964).

6. Wolff, M.E., Burger's Medicinal Chemistry. John Wiley and Sons Ltd., New York, Vol. I. 407 (1980).

7. Franke, R., Theoretical Drug Design Methods, Elsevier Science Publishers, Amsterdam, Vol. VII, 160, 256 (1984).

8. Hein, D.W., Alheim, R.J., Leavitt, J.J., "The use of polyphosphoric acid in the synthesis of 2-aryl and 2-arkyl substituted benzimidazoles, benzoxazoles and benzothiazoles" *J. Am. Chem. Sac,* 79, 427-429 (1957).

9. Yalçın, İ., Ören, İ., Şener, E., Akın, A., Uçartürk, N., "The synthesis and the structure-activity relationships of some substituted benzoxazoles, oxazolo (4, 5-b) pyridines, bezothiazoles and benzimidazoles as antimicrobial agents", *Eur. J. Med. Chem.,* 27, 401-406 (1992).

10. Yalçın, 1., Şener, E., Özden, T., Özden, S., Akın, A., "Synthesis and microbiological activity of 5-methyl-2-(p-substitedphenyl) benzoxazoles", *Eur. J. Med. Chem.,* 25, 705-708 (1990).

11. Şener, E., Yalçın, 1., Özden, S., Özden, T., Akın, A., Yıldız, S., "Synthesis and antimicrobial activities of 5-amino-2-(p-substitutedphenyl) benzoxazole derivatives", *Doğa. Bil. Der.,* 11 (3), 391-395 (1987).

12. Şener, E., Turgut, H., Yalçın, 1., Ören, 1., Türker, L., Çelebi, N., Akın, A., "Structure-activity relationships of some antimicrobial 5-substituted-2-(3-pyridil) benzoxoazoles using quantum-chemical calculations", *İntern. J. Pharm.* 110, 109-115 (1994).

13. Hasebe, N., "Use of polyphosphoric acid in synthesis of 2-aryl-substituted benzoxazoles", *Yamagata Daigaku Kiyo. Shizen Kagaku,* 8(4), 471-477, 1957, Ref: CA: 84: 43918g (1976).

14. Shadomy, S., Espinel, A., In: Manual of Clinical Microbiology Am. Soc. Microb. Washington DC. 3rd edn. pp: 647 (1980).

15. Charles, E.E., Agrawal, V.K., Sharma, S., Iyer, R. N.,"Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents", *Eur. J. Med. Chem.-Chimica Therapeutica,* 14, 435-138 (1979).