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ANTIFUNGAL ACTIVITY OF 2-BENZYLBENZOXAZOLE DERIVATIVES AND QSARs BY FREE-WILSON ANALYSIS

Esin ŞENER** **İsmail YALÇIN***

Ahmet AKIN** **Ningur NOYANALPAN*****

Key Word Index

5 - Substituted-2-(p-substituted - benyl)benzoxazoles, Antifungal activity, **C. albicans**, Free-Wilson, QSAR.

ABSTRACT

The antifungal activity of 5-substituted-2-(p-substituted-benzyl) benzoxazole derivatives against **C. albicans** was determined using progressive double dilution tecnique. The compounds were found significantly active (MIC : 6.2 - 12.5 μg/ml).

The quantitative structure-activity relationships (QSAR) of the compounds were studied using the Free-Wilson approach. The structural parameters were used in the multiple regression analysis.

The results that we had obtained from the QSAR analysis suggest that the 5th position of 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-NO_2 and p-Br groups are the most favourable substituents among the others.

(*) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, TURKEY.

(**) Department of Microbiology, Faculty of Pharmacy, Ankara University, Ankara, TURKEY.

(***) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, TURKEY.

INTRODUCTION

2-Substituted benzoxazole derivatives were prominently studied trusting that the 2nd position is decisive for the biological activity[1,9], whereas position 5[3,5,9,10] prevaling the intensity of the activity. In the previous papers, the synthesis and structure elucidations of 5-substituted-2-(p-substituted-benzyl)benzoxazoles were given[11-13]. These 14 compounds carry H, OCH_3, Cl, Br, NO_2 groups at the para position (Table 1).

Some pharmacological activities of 2-benzylbenzoxazoles have been studied among which antiinflammatory[14,15], antivirutic[16], anticonvulsant[1], antihistaminic[11-13] and the activity against serin proteinase[17] can be mentioned. Antifungal activity of these derivatives have not been studied before. Regarding the statement by Davis at al. that 5 membered heterocycles possessing two benzene rings exhibit chemotherapeutic activity[18], it has been decided to study the antifungal activity of 2-benzylbenzoxazoles that posses one phenyl fused with oxazole and the other attached to position 2.

The Free-Wilson approach is a satisfactory method applied for quantitative structure-activity relationships[19,20]. The basic assumption of this procedure 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 the segments involved.

Like Hansch analysis, Free-Wilson analysis can be applied to homologous series where only substituents are varied in a constant molecule[21,22]. In this study, 2-benzylbenzoxazole has been chosen as the constant molecule. Substituting this molecule at the 5th and the para positions two-dimensional set of congeners has been obtained. Using these structural parameters as molecular desriptors in Free-Wilson analysis, the most favourable substituents have been searched. It has also been tried to find out the most significant position for antifungal activity against **C. albicans.**

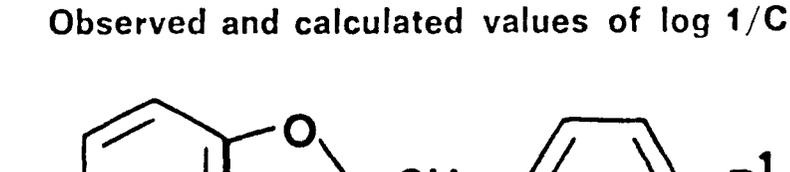
RESULTS and DISCUSSION

For QSAR studies using the Free-Wilson model, the antifungal activity against **C. albicans** was chosen as the biological activity. The

antifungal activities of 2-benzylbenzoxazole derivatives were tested and the compounds were found significantly active (MIC : 12.5 - 6.2 μg/ml) which have not been reported before.

Table 1 indicates that the statistical data are extremely good providing an excellent fit at a very high level of significance with the biological data being explained by the activity contributions having

TABLE — 1. The antifungal activity of the compounds (MIC : μg/ml). Observed and calculated values of log 1/C.



Comp. no	R^1	R^2	MIC (μg/ml)	log 1/C Observed	log 1/C Calculated	Residual
1	H	H	12.5	4.224	4.230	—0.006
2	OCH_3	H	12.5	4.282	4.283	—0.001
3	Br	H	12.5	4.363	4.357	0.006
4	Cl	H	12.5	4.290	4.290	0.000
5	NO_2	H	12.5	4.308	4.307	0.001
6	H	Cl	12.5	4.290	4.288	0.002
7	OCH_3	Cl	12.5	4.340	4.340	0.000
8	Br	Cl	12.5	4.412	4.414	—0.002
9	NO_2	Cl	12.5	4.364	4.364	0.000
10	H	NO_2	6.2	4.609	4.605	0.004
11	OCH_3	NO_2	6.2	4.658	4.657	0.001
12	Br	NO_2	6.2	4.727	4.731	—0.004
13	Cl	NO_2	6.2	4.665	4.665	0.000
14	NO_2	NO_2	6.2	4.680	4.681	—0.001

very good R^2, P value and standart deviations. The differences between calculated and observed log 1/C values have been very small and outliers did not exist.

Table 2 shows the activity contributions of the substituents at both positions. The most favourable substituents in these series are Br as R^1 and NO_2 as R^2 groups. μ value shows the value of log 1/C for the unsubstituted compound.

TABLE — 2. Activity contributions and statistical data for the compounds against C. albicans.

R^1		R^2	
a_{11} (H)	= —0.0831	a_{21} (H)	= —0.1501
a_{12} (OCH_3)	= —0.0109	a_{22} (Cl)	= —0.0928
a_{13} (Br)	= 0.0631	a_{23} (NO_2)	= 0.2240
a_{14} (Cl)	= —0.0033		
a_{15} (NO_2)	= 0.0131		

μ = 4.4437

n = 14, R^2 = 0.9998, s = 0.0043, F = 3832.8 ($P < 0.006 \times 10^{-8}$)

The range of the activity contribution values for the substitution site provides information about the sensitivity of biological activity to the variation of substituents in that position[22]. For our example the equations are :

$$R^2 = a_{23} - a_{21} = 0.2243 + 0.1501 = 0.3744 \quad (1)$$

$$R^1 = a_{13} - a_{11} = 0.0631 + 0.0631 = 0.1262 \quad (2)$$

Although, it appears that two positions of 2-benzylbenzoxazole derivatives have additive effects for the antifungal activity, the 5 position has much more significance than the para position.

Groups like —CN, —CHO, —SO_2CH_3 can be regarded as future candidates to replace —NO_2 as they have similar π, F, R and MR values[22,23].

EXPERIMENTAL

Antifungal Activity

The activity of the compounds against **Candida albicans** RSKK 628 were tested in Sabouraud's broth. 0.2 ml of fungal culture has been inoculated into broth and the medium was incubated for 5 days at 25°C. Progressive double dilution technique was applied in a dilution serial of 400, 200, 100, 50, 25, 12.5, 6.2, 3.1, 1.5 and 0.7 μg ml. A set of tubes containing only inoculated broth was kept as controls. After incubation for 5 days, the first tube with no growth of the fungus was taken to represent the minimum inhibitory concentration (MIC, expressed in μg/ml).

The activities of the compounds were tested in absolute alcohol[24]. For that reason, the activity of ethyl alcohol against **C. albicans** has been tested in the same dilutions and found inactive. The antifungal activities of the compounds were given in Table 1.

The log 1/C values were used in the Free-Wilson analysis, where C. denoted the molar concentrations of the MIC values of the compounds.

Free-Wilson Analysis

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 the substituents. Quantitatively, Free-Wilson additivity model is given by the equation below[21] :

biological activity = sum of group contributions + overall average activity where this model is described by the equation 3 :

$$\log 1/C = \Sigma a_i x_i + \mu \qquad (3)$$

where a_i is the contribution of the i^{th} substituent, and if the substituent is present in the molecule, x_i has a value of 1, otherwise a value of 0. The overall average activity calculated for the unsubstituted compound is indicated as μ.

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

matrix has been used in equation 3 and yielded the linear equation system below :

TABLE — 3. Structure matrix of the compounds derived from Free-Wilson model.

	R^1					R^2		
	a_{11}	a_{12}	a_{13}	a_{14}	a_{15}	a_{21}	a_{22}	a_{23}
Comp. no	**H**	**OCH_3**	**Br**	**Cl**	**NO_2**	**H**	**Cl**	**NO_2**
1	1	0	0	0	0	1	0	0
2	0	1	0	0	0	1	0	0
3	0	0	1	0	0	1	0	0
4	0	0	0	1	0	1	0	0
5	0	0	0	0	1	1	0	0
6	1	0	0	0	0	0	1	0
7	0	1	0	0	0	0	1	0
8	0	0	1	0	0	0	1	0
9	0	0	0	0	1	0	1	0
10	1	0	0	0	0	0	0	1
11	0	1	0	0	0	0	0	1
12	0	0	1	0	0	0	0	1
13	0	0	0	1	0	0	0	1
14	0	0	0	0	1	0	0	1

TABLE — 4. Correlation matrix derived from symmetry equations.

		R^1			R^2	
	a_{12}	a_{13}	a_{14}	a_{15}	a_{22}	a_{23}
Comp. no	OCH_3	Br	Cl	NO_2	Cl	NO_2
1	—1	—1	—0.667	—1	—0.8	—1
2	1	0	0	0	—0.8	—1
3	0	1	0	0	—0.8	—1
4	0	0	1	0	—0.8	—1
5	0	0	0	1	—0.8	—1
6	—1	—1	—0.667	—1	1	0
7	1	0	0	0	1	0
8	0	1	0	0	1	0
9	0	0	0	1	1	0
10	—1	—1	—0.667	—1	0	1
11	1	0	0	0	0	1
12	0	1	0	0	0	0
13	0	0	1	0	0	1
14	0	0	0	1	0	1

$$
\begin{aligned}
&1)\ \log 1/C = a_{21} + a_{11} + \mu = 4.224\\
&2)\ \log 1/C = a_{21} + a_{12} + \mu = 4.282\\
&3)\ \log 1/C = a_{21} + a_{13} + \mu = 4.363\\
&4)\ \log 1/C = a_{21} + a_{14} + \mu = 4.290\\
&5)\ \log 1/C = a_{21} + a_{15} + \mu = 4.308\\
&6)\ \log 1/C = a_{22} + a_{11} + \mu = 4.290\\
&7)\ \log 1/C = a_{22} + a_{12} + \mu = 4.340\\
&8)\ \log 1/C = a_{22} + a_{13} + \mu = 4.412
\end{aligned}
\qquad (4)
$$

9) $\log 1/C = a_{22} + a_{15} + \mu = 4.364$

10) $\log 1/C = a_{23} + a_{11} + \mu = 4.609$

11) $\log 1/C = a_{23} + a_{12} + \mu = 4.658$

12) $\log 1/C = a_{23} + a_{13} + \mu = 4.727$

13) $\log 1/C = a_{23} + a_{14} + \mu = 4.665$

14) $\log 1/C = a_{23} + a_{15} + \mu = 4.680$

Additional restrictive equations, so called symmetry conditions have been formulated so that for each position the sum of the varying groups equaled to 0[19,22]. The symmetry conditions for our sample have been :

$$5a_{21} + 4a_{22} + 5a_{23} = 0 \quad (5)$$

$$3a_{11} + 3a_{12} + 3a_{13} + 2a_{14} + 3a_{15} = 0 \quad (6)$$

a_{21} and a_{11} have been selected at each position as a dependent variable from the equations 5 and 6.

$$a_{21} = -4/5\, a_{22} - a_{23} \quad (7)$$

$$a_{11} - a_{12} - a_{13} - 2/3\, a_{14} - a_{15} \quad (8)$$

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.

Regression analysis equation of the QSAR study has been performed by using IBM-XT personal computer working with Microstat Statistic Package.

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