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The Antibacterial Activity Of 2-Phenyloxazolo (4,5-b) Pyridine Derivatives Against Gram (-) Bacteria And The Quantitative Structure-Activity Relationships

İsmail YALÇIN (*)
Esin ŞENER (*)
Tuncel ÖZDEN (***)
Ahmet AKIN (**)
Sulhiye YILDIZ (**)

Summary : The antibacterial activity of 2-(p-substituted-phenyl) oxazolo(4,5-b)pyridine derivatives against some Gram (—) bacteria were determined using the Dilution Technique. The compounds were found significantly active (MIC : 3.1-25 μg/ml).

The quantitative structure-activity relationships (QSAR) of the compounds were studied using some hydrophobic (π, π^2), electronic (δ, F, R) and steric (MR, MW, P_r) physicochemical parameters. The correlation equations of these relationships which were designed according to the Hansch Analysis Method were given.

For the correlation of the antibacterial activity against Gram (—) bacteria with the molecular criteria in the series of 2-phenyloxazolo (4,5-b)pyridines, steric effects were found more significant as compared to hydrophobic and electronic effects.

(*) A.Ü. Eczacılık Fakültesi, Farmasötik Kimya Anabilim Dalı, Tandoğan - Ankara.

(**) A.Ü. Eczacılık Fakültesi, Mikrobiyoloji Bilim Dalı, Tandoğan - Ankara.

(***) Karadeniz Teknik Üniversitesi - Trabzon.

2—FENİL—OKSAZOLO(4,5-b)PİRİDİN TÜREVLERİNİN GRAM (—) BAKTERİLERE KARŞI ANTİBAKTERİYEL ETKİLERİ VE KANTİTATİF YAPI—ETKİ İLİŞKİLERİ

Özet : 2-(p-Sübstitüe-fenil)oksazolo(4,5-b)piridin türevlerinin baz Gram (—) bakterilere karşı antibakteriyel etkileri Tüpte Dilüsyon yön temi ile saptanmıştır. Bileşikler dikkate değer antibakteriyel etkili bu lunmuşlardır (MIC = 3.1-25 μg/ml).

Bileşiklerin kantitatif yapı-etki ilişkileri bazı hidrofobik (π, π^2 elektronik (δ, F, R) ve sterik (MR, MW, P_r) özellikteki fizikokimyas parametreler kullanılarak çalışılmıştır. Hansch Analiz yönteminde faydalanılarak bulunan korrelasyon denklemleri verilmiştir.

2-Feniloksazolo(4,5-b)piridin türevlerinde Gram (—) bakterilere kar aktivite ile bileşiklerin sterik özellikleri arasındaki ilişkinin, hidrofob ve elektronik özelliklerle olan ilişkilere göre daha anlamlı olduğu c taya konulmuştur.

Keywords : 2-Phenyloxazolo(4,5-b)pyridines, Gram (—) Bacter π, π^2, δ, F, R, MR, MW, P_r, QSAR.

INTRODUCTION

Quantitative drug design can contribute both to the discovery of new therapeutic agents and to the progress of biomedical research in general. Devoloping techniques are becoming increasingly more capable of directing synthetic effort from compounds that have a low probabilty of success. From that phenomenon, we decided to determine the antibacterial activity of 2-(p-substituted-phenyl)-oxazolo (4,5-b)pyridine derivatives against some (Gram (—) bacteria and study the quantitative structure-activity relationships (QSAR) of these compounds in order to design of more active compounds in these series. In our previous papers, syn and structure elucidations of substituted-phenyl)oxazolo(4,5 ridine derivatives were sta and the quantitative stru activity relationships of these pounds in Gram (+) bacteria C. albicans were reported (7 this research, the antibacter tivity of these compounds Gram (—) bacteria are thou the function of the physicc cal parameters on lead optim method.

For our QSAR studies w some steric, electronic and phobic parameters which ar in Table 2. The multiple re analysis method is used w

volves finding the best fit of a dependent variable (the microbiological activity) to a linear combination of the independent variables (descriptors) by the method of least squares. This is formally expressed as follows;

$y = a_0 + a_1x_1 + a_2x_2 + \ldots\ldots\ldots + a_nx_n$

where x_1, x_2, ... x_n are the descriptor values (physicochemical substituent constants), y is related to the microbiological activity of oxazolopyridine derivatives, and a_0, a_1, ... a_n are the coefficients determined by least squares analysis. This equation is developed for each oxazolopyridine derivative in our analysis.

MATERIAL AND METHOD

Microbiology

For antimicrobial activity the Gram (—) bacteria below were chosen;

1 — Escherichia coli ATCC 10536

2 — Klebsiella pneumoniae NTCC 52211

3 — Pseudomonas aeruginosa RSKK 628

The microbiological activity were tested as described earlier (2). All the bacteria were prepared in Nutrient broth (Beef extract : 3 g, Peptone : 5 g, Sodium Chloride : 5 g, Agar : 5 g, Distilled water : 1000 ml). Testing was done in Mueller Hinton broth (Meet infusion : 6 g, Casein hydrolysate : 17.5 g, Starch : 1.5 g, Distilled water : 1000 ml). The twofold serial dilution technique was applied. After inoculation with 0.2 ml of culture from the nutrient broth, the seeded broths were incubated 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 tupe with no growth of the microorganism was taken to represent the minimum inhibitory concentration (MIC, expressed in μg/ml).

The antimicrobial activity of Amoxicillin, Ampicillin, Chloramphenicol and Erithromycin against the same microorganisms were tested using the same methods and MIC values were given. The activity of the compounds were tested in absolute ethanol (4,5). For that reason, the activity of ethanol against the same microorganisms were tested in the same dilutions and found inactive. The antimicrobial activity of the compounds were given in Table 1.

Determination of the Parameters

Regression analysis equations of the QSAR studies were performed using IBM-XT computer working with Microstat Statistic Package. Parachor (P_r) values of each compound were calculated by the additive summation of the P values of all the atoms and the structural features using Qayle's Table (6). π, δ, F, R, MR and MW values were taken from the table given by Hansch et al. (7). These values were shown in Table 2.

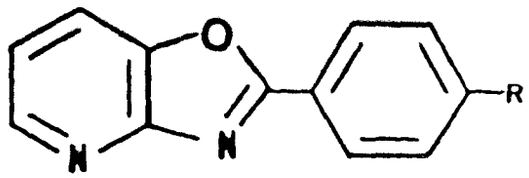

Table 1 : 2-Phenyloxazolo(4,5-b)pyridine derivatives and their antibacterial activities (MIC in μg/ml)

Comp. no	R	E. coli	K. pneumoniae	P. aeruginosa
1	CH_3	25.0	12.5	12.5
2	C_2H_5	12.5	6.2	12.5
3	$C(CH_3)_3$	25.0	12.5	12.5
4	OCH_3	6.2	6.2	12.5
5	OC_2H_5	12.5	6.2	12.5
6	NH_2	12.5	3.1	12.5
7	NO_2	25.0	6.2	12.5
8	Cl	12.5	3.1	12.5
9	Br	12.5	6.2	12.5
Ampicillin		1.5	12.5	500.0
Amoxicillin		1.5	12.5	1000.0
Chloramphenical		25.0	12.5	25.0
Erithromycin		50.0	50.0	25.0

Table 2 : The physicochemical parameters of 2-phenyloxazolo(4,5-b)pyridine derivatives

Comp. no	π	π^2	δ	F	R	MR	MW	P_r
1	0.56	0.3136	—0.17	—0.04	—0.13	5.65	15.0	412.5
2	1.02	1.0404	—0.15	—0.05	—0.10	10.30	29.1	452.5
3	1.98	3.9204	—0.20	—0.07	—0.13	19.62	57.1	532.5
4	—0.02	0.0004	—0.27	0.26	—0.51	7.87	31.4	443.4
5	0.38	0.1444	—0.24	0.22	—0.44	12.47	45.1	483.4
6	—1.23	1.5129	—0.66	0.02	—0.68	5.42	16.0	403.2
7	—0.28	0.0784	0.78	0.67	0.16	7.36	46.0	417.8
8	0.71	0.5041	0.23	0.41	—0.15	6.03	35.4	415.9
9	0.86	0.7396	0.23	0.44	—0.17	8.88	79.9	428.7

Table 3 : Antibacterial activity of 2-phenyloxazolo(4,5-b)pyridine derivatives (log 1/C).

Com. No	P. aeruginosa Obsd	Calcd*	Resid-ual	E. coli Obsd	Calcd**	Resid-ual	K. pneumoniae Obsd	Calcd***	Resid-ual
1	4.226	4.226	0.000	3.925	3.928	—0.003	3.925	—	—
2	4.254	4.253	0.001	4.254	4.249	0.005	4.254	4.255	—0,001
3	4.305	4.305	0.000	4.004	4.005	—0.001	4.305	4.304	0,001
4	4.258	4.258	0.000	4.258	4.254	0.002	4.258	4.258	0,000
5	4.284	4.284	0.000	4.284	4.287	—0.003	4.284	4.283	0,001
6	4.228	4.228	0.000	4.228	4.226	0.002	4.532	—	—
7	4.285	4.285	0.000	3.984	3.981	0.003	4.285	4.284	0,001
8	4.266	4.266	0.000	4.266	4.273	—0.007	4.571	—	—
9	4.343	4.343	0.000	4.343	4.342	0.001	4.343	4.344	—0,001

Calculated from *eq. 5, **eq. 15, ***eq. 25.

RESULTS AND DISCUSSION

Antibacterial activities of 2-phenyloxazolo(4,5-b)pyridines against some Gram (—) bacteria such as E. coli, K. pneumoniae, P. aeruginosa were tested and they were found significantly active (MIC : 3.1-25 μg/ml). Two compounds (Comp. no : 6,8) were the most active derivatives in their series (MIC : 3.1 μg/ml) in K. pneumoniae. For QSAR studies some hydrophobic (π, π^2), electronic (δ, F, R) and steric (MR, MW, P_r) parameters used. The lead optimization method has been examined by multiple regression analysis using the Microstat computer program. Log 1/C values were used in the regression equations, where C was the molar concentrations of the MIC values of the compounds.

From the data in Tables 2 and 3, eq. 1-9 for the antimicrobial activity of 2-phenyloxazolo(4,5-b)pyridine derivatives against P. aeruginosa have been derived. The forward and backward stepwise developments of eq. 5 are stated below :

$$\log 1/C = 0.02\ (\pm 0.01)\ \pi + 4.26 \quad (1)$$
$$n = 9,\ R^2 = 0.2460,\ s = 0.03,\ F = 0.17$$
$$\log 1/C = 0.02\ (\pm 0.01)\ \pi + 0.04\ (\pm 0.03)\ \delta + 4.27 \quad (2)$$
$$n = 9,\ R^2 = 0.4236,\ s = 0.03,\ F = 2.2$$
$$\log 1/C = 0.04\ (\pm 0.01)\ \pi - 0.06\ (\pm 0.05)\ \delta + 0.17\ (\pm 008)\ F + 4.22 \quad (3)$$
$$n = 9,\ R^2 = 0.7035,\ s = 0.03,\ F = 3.95$$
$$\log 1/C = 0.02\ (\pm 0.02)\ \pi - 0.04\ (\pm 0.05)\ \delta + 0.16\ (\pm 0.08)\ F + 0.004\ (\pm 0.003)\ MR + 4.19 \quad (4)$$

$n = 9, R^2 = 0.7909, s = 0.02, F = 3.78$

$$\log 1/C = 0.0011\ (\pm 0.0006)\ \pi - 0.0019\ (\pm 0.0016)\ \delta + 0.0124\ (\pm 0.0032)\ F + 0.0005\ (\pm 0.0001)\ MR + 0.0017\ (\pm 0.00003)\ MW + 4.1981 \quad (5)$$

$n = 9, R^2 = 0.9999, s = 0.0007, F = 4091.696\ (P = 0.000006)$

$$\log 1/C = 0.002\ (\pm 0.00003)\ MW + 0.0006\ (\pm 0.0001)\ MR + 0.008\ (\pm 0.003)\ F + 0.00006\ (\pm 0.001)\ \delta + 4.2 \quad (6)$$

$n = 9, R^2 = 0.9997, s = 0.0009, F = 3421.22$

$$\log 1/C = 0.002\ (\pm 0.00002)\ MW + 0.0006\ (\pm 0.0001)\ MR + 0.009\ (\pm 0.001)\ F + 4.2 \quad (7)$$

$n = 9, R^2 = 0.0007, s = 0.0008, F = 5699.12$

$$\log 1/C = 0.002\ (\pm 0.00004)\ MW + 0.0002\ (\pm 0.0002)\ MR + 4.2 \quad (8)$$

$n = 9, R^2 = 0.9983, s = 0.002, F = 1785.61$

$$\log 1/C = 0.002\ (\pm 0.00003)\ MW + 4.2 \quad (9)$$

$n = 9, R^2 = 0.9979, s = 0.002, F = 3391.53$

The negative coefficient with in eq. 5 denote that this parameter has negative effect for the activity against P. aeruginosa. From the correlation matrix it is found that MW is the most suitable parameter for the activity. The correlation of MW with log 1/C shows high degree of correlation ($R^2 = 0.9979$) and the P value of the F test is found as 0.01×10^{-8} which shows us that MW is highly related to log 1/C in the regression analysis (eq. 9). The increase of the activity is proportional with the increase of the molecular weights (MW) of the substituents in the para position.

Correlation matrix between regression parameters and log 1/C for eq. 1-9 in P. aeruginosa is :

	log 1/C	π	π^2	δ	F	R	MR	MW	P_r
log 1/C	1.000								
π	0.496	1.000							
π^2	0.222	0.509	1.000						
δ	0.487	0.143	—0.313	1.000					
F	0.461	—0.263	—0.541	0.815	1.000				
R	0.351	0.488	0.014	0.831	0.365	1.000			
MR	0.510	0.717	0.742	—0.119	—0.336	0.133	1,000		
MW	0.998	0.487	0.222	0.475	0.451	0.342	0,494	1,000	
P_r	0.423	0.695	0.655	—0.195	—0.368	0.042	0,980	0,403	1,000

Eq. 10-20 have been derived from the data in Tables 2 and 3 for the antibacterial activity of the compounds against E. coli. When the combination of electronic, steric and hydrophobic parameters are used δ and MW are found more suitable than others that these parameters have positive effects for the activity which is the same in P. aeruginosa. The forward and backward stepwise developments of eq. 15 are given below :

$$\log 1/C = -0.03\ (\pm 0.05)\ \pi^2 + 4.20 \quad (10)$$

$n = 9, R^2 = 0.0650, s = 0.16, F = 0.49$

$$\log 1/C = -0.05\ (\pm 0.05)\ \pi^2 - 0.12\ (\pm 0.15)\ \delta + 4.20 \quad (11)$$

$n = 9, R^2 = 0.1609, s = 0.16, F = 0.58$

$$\log 1/C = -0.01\ (\pm 0.05)\ \pi - 0.39\ (\pm 0.24)\ \delta + 0.58\ (\pm 0.42)\ F + 4.04 \quad (12)$$

$n = 9, R^2 = 0.3936, s = 0.15, F = 1.08$

$$\log 1/C = -0.02\ (\pm 0.06)\ \pi^2 - 21.88\ (\pm 20.68)\ \delta + 20.52\ (\pm 19.19)\ F + 21.41\ (\pm 20.59)\ R + 3.97 \quad (13)$$
$$n = 9,\ R^2 = 0.5226,\ s = 0.15,\ F = 1.10$$

$$\log 1/C = -0.50\ (\pm 0.14)\ \pi^2 + 300.97\ (\pm 91.42)\ \delta - 282.63\ (\pm 85.82)\ F + 300.42\ (\pm 91.12)\ R + 0.06\ (\pm 0.02)\ MW + 4.00 \quad (14)$$
$$n = 9,\ R^2 = 0.9084,\ s = 0.08,\ F = 5.95$$

$$\log 1/C = -0.5641\ (\pm 0.0153)\ \pi^2 + 355.27\ (\pm 10.26)\ \delta - 333.74\ (\pm 9.63)\ F - 354.54\ (\pm 10.22)\ R + 0.0707\ (\pm 0.0019)\ MW - 0.0018\ (\pm 0.0001)\ P_r + 4.7568 \quad (15)$$
$$n = 9,\ R^2 = 0.9993,\ s = 0.008,\ F = 484.79\ (P = 0.002)$$

$$\log 1/C = -0.0008\ (\pm 0.002)\ P_r + 0.002\ (\pm 0.01)\ MW + 11.83\ (\pm 53.45)\ R + 11.53\ (\pm 50.08)\ F - 12.29\ (\pm 53.58)\ \delta + 4.25 \quad (16)$$
$$n = 9,\ R^2 = 0.5300,\ s = 0.17,\ F = 0.68$$

$$\log 1/C = -0.001\ (\pm 0.0002)\ P_r + 0.04\ (\pm 0.004)\ MW - 0.43\ (\pm 0.23)\ R + 0.04\ (\pm 0.32)\ F + 4.32 \quad (17)$$
$$n = 9,\ R^2 = 0.5217,\ s = 0.15,\ F = 1.10$$

$$\log 1/C = 0.001\ (\pm 0.001)\ P_r + 0.005\ (\pm 0.003)\ MW - 0.42\ (\pm 0.20) + 4.39 \quad (18)$$
$$n = 9,\ R^2 = 0.5194,\ s = 0.14,\ F = 1.80$$

$$\log 1/C = -0.0008\ (\pm 0.002)\ P_r + 0.003\ (\pm 0.003)\ MW + 4.44 \quad (19)$$
$$n = 9,\ R^2 = 0.0999,\ s = 0.17,\ F = 0.33$$

$$\log 1/C = -0.0003\ (\pm 0.001)\ P_r + 4.32 \quad (20)$$
$$n = 9,\ R = 0.0081,\ s = 0.17,\ F = 0.06$$

Correlation matrix between the regression parameters and log 1/C for eqs. 10-20 in E. coli is :

	log 1/C	π	π^2	δ	F	R	MR	MW	P_r
log 1/C	1.000								
π	—0.133	1.000							
π^2	—0.255	0.509	1.000						
δ	—0.214	0.143	—0.314	1.000					
F	0.166	—0.264	—0.541	0.815	1.000				
R	—0.501	0.489	0.014	0.832	0.357	1.000			
MR	—0.134	0.718	0.742	—0.120	—0.336	0.134	1,000		
MW	0.241	0.488	0.223	0.476	0.452	0.342	0,494	1,000	
P_r	—0.090	0.696	0.655	—0.196	—0,369	0,042	0,981	0,404	1,000

After finding out the significant relationship of MW and the activity of the compounds against P. aeruginosa we tried out the effect of the same parameter in E. coli. When 3 data points in Tables 2 and 3 (Comp. no 1, 3, 7) have not been used in this equation, the square of regression coefficient became as 0.9983 and $P = 0.1 \times 10^{-5}$ (eq. 21).

$$\log 1/C = 0.0018\ (+ 0.00003)\ MW + 4.2018 \quad (21)$$
$$n = 6,\ R^2 = 0.9983,\ s = 0.0018,\ F = 2364.55,\ P = 0.1 \times 10^{-5}$$

Eq. 22-27 have been derived from the same data given in Tables 2 and 3 for the antibacterial activity of the compounds against K. pneumoniae. Although R^2 is found as 0.9692 the P value of the F-test for the best equation is predicted os 0.0895 which shows us that the physicochemical parameters

used as independent variables are not exactly related to the dependent variable. The equation is given below :

$$\log 1/C = -0.4132\ (\pm 0.122)\,\pi^2 + 383.30\ (\pm 81.95)\,\delta - 359.2\ (\pm 76.96)\ F - 382.5\ (\pm 81.69)\ R + 0.0686\ (\pm 0.0152)\ MW - 0.0023\ (\pm 0.0009)\ P_r + 5.0462 \quad (22)$$

$n = 9,\ R^2 = 0.9692,\ s = 0.065,\ F = 10.501,\ P = 0.0895$

Consequently, we eliminated 3 data points in Tables 2,3 (Comp. no 1, 6, 8) and derived eq. 25.

$$\log 1/C = -0.03\ (\pm 0.05)\ F + 4.27 \quad (23)$$

$n = 6,\ R^2 = 0.0886,\ s = 0.03,\ F = 0.39$

$$\log 1/C = 0.09\ (\pm 0.07)\ F + 0.005\ (\pm 0.004)\ MR + 4.19 \quad (24)$$

$n = 6,\ R^2 = 0.4219,\ s = 0.03,\ F = 1.10$

$$\log 1/C = 0.0057\ (\pm 0.0007)\ F + 0.0004\ (\pm 0.00004)\ MR + 0.0017\ (\pm 0.000008)\ MW + 4.2003 \quad (25)$$

$n = 6,\ R^2 = 1.0000,\ s = 0.0003,\ F = 29502.1,\ P = 0.00003$

$$\log 1/C = 0.001\ (\pm 0.00003)\ MW + 0.0001\ (\pm 0.0001)\ MR + 4.2029 \quad (26)$$

$n = 6,\ R^2 = 0.9992,\ s = 0.001,\ F = 1871.4$

$$\log 1/C = 0.001\ (\pm 0.00003)\ MW + 4.20 \quad (27)$$

$n = 6,\ R^2 = 0.9989,\ s = 0.001,\ F = 3593.4,\ P = 0.05 \times 10^{-5}$

Correlation matrix of the regression parameters and log 1/C for 23-27 in K. pneumoniae is :

	log 1/C	π	π^2	δ	F	R	MR	MW	P_r
log 1/C	1.000								
π	0.339	1.000							
π^2	0.288	0.918	1.000						
δ	0.318	—0.448	—0.289	1.000					
F	0.298	—0.761	—0.642	0.827	1.000				
R	0.223	0.074	0.210	0.793	0.312	1.000			
MR	0.204	0.849	0.905	—0.474	—0.699	—0.040	1,000		
MW	0.999	0.337	0.277	0.312	0.296	0.216	0,187	1,000	
P_r	0.043	0.761	0.809	—0.628	—0.757	—0.238	0,972	0,026	1,000

In eq. 25 one can see the importance of steric factors (MR and MW) in K. pneumoniae. Although F as an electronic parameter takes place in that equation, it has not showed an important role due to eq. 23 in the stepwise development of eq. 25. MW has a high positive coefficient than MR (eq. 25, 26) which suggests that it is the favourable parameter for the activity. The prediction of R^2 as 0.9989 ($P = 0.05 \times 10^{-5}$) in the relationship between MW and the activity (eq. 27) makes clear that the molecular weights of the substituents in the para position have significant contribution to the estimation of the activity.

From the results, it can be concluded that for the correlation of the antibacterial activity against Gram (—) bacteria in the series of 2-phenyloxazolo (4,5-b)pyridines, steric effects are more merited as compared to hydrophobic and electronic effects.

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