Int. J. Mol. Sci. **2006**, *7*, 119-129

International Journal of
Molecular Sciences
ISSN 1422-0067

www.mdpi.org/ijms/

QSAR of Some N^1-Aryl/Heteroarylaminomethyl/ethyl-1,2,4-Triazoles Part II: Antimicrobial Activity Against *Bacillus Subtilis*

Vesna Dimova [1,*], Katica Colanceska Ragenovic [1] and Vladimir Kakurinov [2]

1 Faculty of Technology and Metallurgy, The "Sv. Kiril & Metodij" University, Ruger Boskovic 16, 1000 Skopje, Macedonia
2 Faculty of Agriculture, Department of Microbiology, The "Sv. Kiril & Metodij" University, bul. Aleksandar Makedonski, bb, 1000 Skopje, Macedonia

* Author to whom correspondence should be addressed; E-mail: vdimova@tmf.ukim.edu.mk

Received: 27 December 2004 / Accepted: 15 August 2005, in Revised Form: 20 April 2006 / Published: 26 April 2006

Abstract: QSAR analysis of a series of previously synthesized N^1-aryl-amino/methyl/ethyl-1,2,4-triazole and N^1-heteroarylamino/methyl/ethyl-1,2,4-triazole derivatives tested for growth inhibitory activity against *Bacillus subtilis*, was performed using the computer-assisted multiple regression procedure. Using the Hansch and Free Wilson approaches the activity contribution for either the aminomethyl/aminoethyl unit or the aromatic/heteroaromatic ring was determined from the correlation equation.

Keywords: QSAR/QSPR; N^1-heteroarylamino/methyl/ethyl-1,2,4-triazole; physico-chemical parameters

1. Introduction

Our society is faced with several challenges that may have a chemical solution. Examples include bacterial drug resistance, new diseases like AIDS, and agricultural pest control. Characterizing the biological activity and properties of all the known compounds is impossible so it is necessary to develop predictive tools for molecular properties and environmental behaviour. Quantitative structure activity relationships (QSAR) and quantitative structure properties relationships (QSPR) play a central role in this effort, so these methods are unquestionably of great importance in modern chemistry and

biochemistry [1-3]. The concept of QSAR/QSPR is to transform searches for compounds with desired properties using chemical intuition and experience into a mathematically quantified and computerized form. Once a correlation between structure and activity/property is found, any number of compounds, including those not yet synthesized, can be readily screened on the computer in order to select structure with the properties desired. It is then possible to select the most promising compounds to be synthesized and tested in the laboratory.

1,2,4-Triazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities. The 1,2,4-triazole nucleus is associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic properties [4-7]. Consequently, spurred by the need for new antimicrobial agents and the fact that many new effective antimicrobial drugs possess heterocyclic rings in their structure, such as a 1,2,4-triazole ring, over the last few years we have synthesized some novel 1,2,4-triazole derivatives and tested them for antibacterial and antifungal effects against *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans* [8-11]. The antibacterial activity of synthesized N^1-aryl/heteroarylamino/methyl/ethyl-1,2,4-triazole derivatives against *Bacillus subtilis* were used in this QSAR analysis.

2. Results and Discussion

In our work, the chosen model is based on the *in vitro* antimicrobial activity of certain N^1-aryl- and N^1-heteroarylaminoaminomethyl/ethyl-1,2,4-triazole derivatives **1**-**18**, (Tables 1 and 2; Figures 1 and 2), against *Bacillus subtilis.*

Table 1. N^1-arylaminomethyl/ethyl-1,2,4-triazole derivatives (**1-10**) used in the present study.

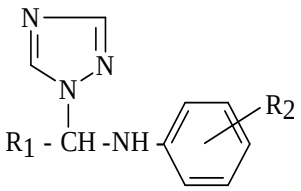
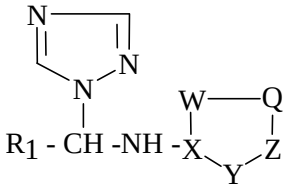


Figure 1.

Comp. No:	***R_1***	***R_2***
1	H	p-$COOC_2H_5$
2	H	p-COOH
3	H	o-COOH
4	H	p-Cl
5	H	p-Br
6	H	p-CH_3
7	H	p-C_6H_5
8	H	p-C_6H_4-CH_2-NH- 
9	CH_3	p-$COOC_2H_5$
10	CH_3	p-NO_2

Table 2. N^1-heteroarylaminomethyl/ethyl-1,2,4-triazole derivatives (**11-18**) used in the present study.

Figure 2.



Comp. No:	R_1	*X*	*Y*	*Z*	*Q*	*W*
11	H	-C=	=N-	-CH=	=CH-	-CH=CH-
12	H	-C=	=N-	-CH=	=CH-	$-C(CH_3)=CH-$
13	H	-C=	=N-	$=C(CH_3)-$	=CH-	-CH=CH-
14	H	-C=	=N-	-CH=	=C(Cl)-	-CH=CH-
15	H	-C=	=N-	-CH=	=N-	-CH=CH-
16	H	-N-	-CH=	=N-	-N=	=CH-
17	CH_3	-C=	=N-	=C(CH3)-	=CH-	-CH=CH-
18	CH_3	-C=	=N-	-CH=	=CH-	-S-

The results of antimicrobial tests indicated that not all compounds exhibited antibacterial and antifungal activities. It must also be noted that compounds **17** and **18** do not inhibit the growth of the selected microorganisms. The inhibitory effects of compounds **1-18**, against *B. subtilis*, expressed as minimum inhibition concentration (MIC), are given in Table 3, where C is the MIC value expressed in molar concentration units (Table 3).

Table 3. Experimentally obtained MIC values, calculated log 1/C and log P.

Compound No:	*MIC*[a]	*log 1/ C*	*log P*[b]	*MR*[c]
1	4.061×10^{-6}	5.3914	*1.2981*	*17.47*
2	2.291×10^{-5}	4.6399	*0.4579*	*6.93*
3	-	-	*1.4690*	*6.93*
4	4.793×10^{-5}	4.3194	*1.2210*	*6.03*
5	1.975×10^{-5}	4.7044	*1.4665*	*8.88*
6	2.656×10^{-5}	4.5757	*1.1238*	*5.65*
7	3.995×10^{-6}	5.3984	*2.3405*	*25.36*
8	-	-	*-1.6900*	-
9	3.842×10^{-5}	4.4154	*1.7157*	*17.47*
10	4.287×10^{-5}	4.3678	*1.3895*	*0.67*
11	5.708×10^{-5}	4.2435	*0.0301*	-
12	-	-	*0.5774*	-
13	5.399×10^{-5}	4.2676	*0.5774*	-
16	6.055×10^{-5}	4.2178	*-2.1800*	-

[a] minimum inhibition concentration expressed in molar concentration; [b] Ref. [12]; [c] Ref. [1].

Using the filter paper disc method [13] compounds **3, 8, 12**, **14** and **15** do not inhibit the growth of the chosen microorganism [10]. From the data obtained, first MIC values were calculated and then the log 1/C values (Table 3). It was important for further analysis to find the correlation matrix for the descriptors used (Table 4).

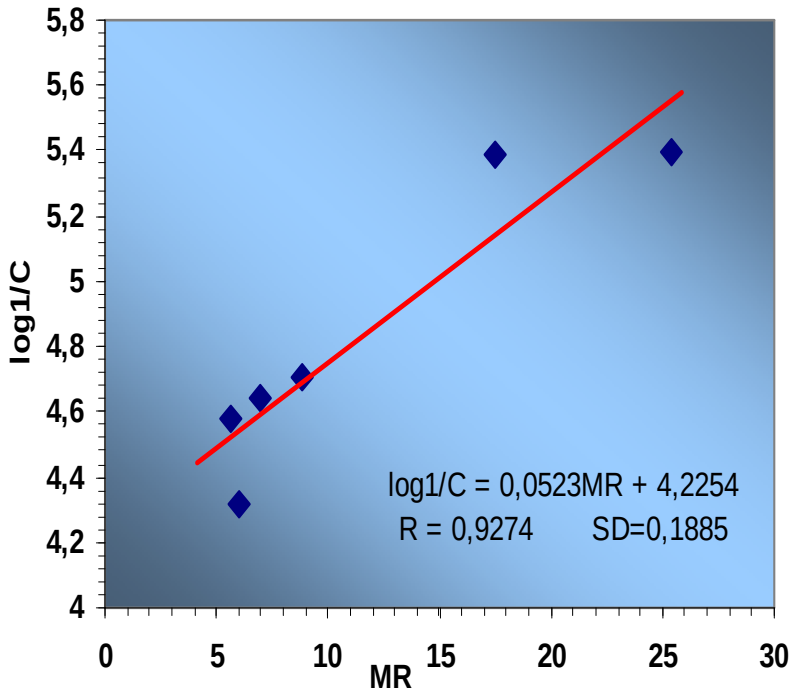
Table 4. Correlation matrix for the chosen electronic, steric and hydrophobic parameters.

	σ	**π**	**logP**	**MR**	**F**	**R**	**L**
σ	1.0000						
π	*-0.8861*	1.0000					
logP	-0.4792	*0.8180*	1.0000				
MR	-0.4751	*0.7235*	*0.7732*	1.0000			
F	0.2547	-0.1186	0.6125	-0.2499	1.0000		
R	0.6327	-0.7356	0.0348	-0.5815	0.3997	1.0000	
L	0.1416	-0.0128	0.4108	0.4519	-0.2317	0.1457	1.0000

In our work, attempts were made to establish a correlation between selected physicochemical properties and experimental values for antimicrobial activities against *B. subtilis*, in three ways:

- *applying the general Hansch equation for structurally identical compounds* (**1**-**8**)*;*
- *using the Free Wilson approach which included derivatives with some structural changes (aminomethyl unit has been replaced with aminoethyl group), compounds* (**1**-**10**);
- *extend the Free Wilson equation, for determination of the influence of heterocyclic ring, substituted on the amino group, compounds* (**1**-**18**).

Figure 3. Linear correlation between log 1/C and MR



When the data in Tables 3 and 7 were submitted to linear regression (Figure 3) the resulting QSAR equation is:

$$\log 1/C = 0.0523MR + 4.2254$$
$$R = 0.9274 \qquad SD=0.1885$$

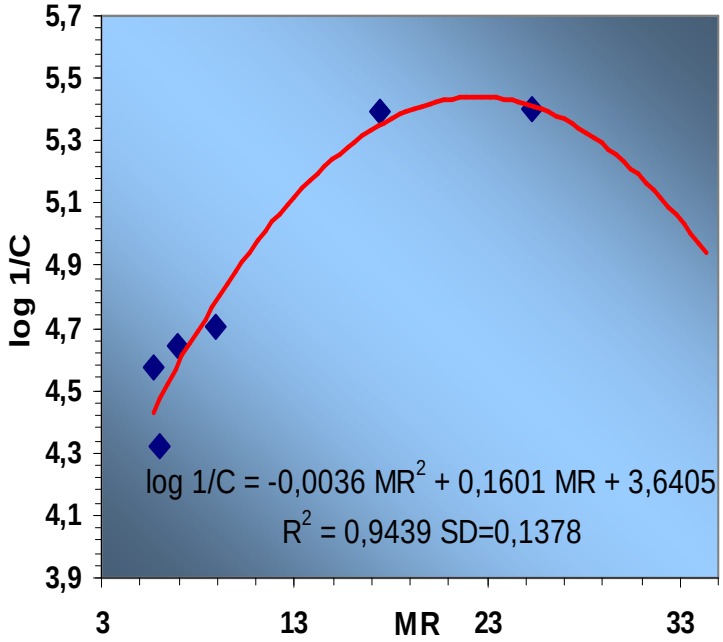
where R is correlation coefficients and SD is the standard deviation.

The data for the chosen compounds were reasonably well correlated with a regression coefficient of 0.9274, indicating a relatively good fit.

However, moderate linear collinearities exist between log1/C and other selected descriptors (σ, π, logP, F, R and L) where R is below 0.7. This weak correlation is unable to describe the biological activities of the selected set of compounds. Addition of some other groups to the parent triazole ring would certainly improve those linear fittings.

An attempt was also made to find the parabolic correlation. Parabolic relationships between biological response (log1/C) and logP term, can be explained by the fact that many membranes must be traversed for compounds to get to the target site, and those with greatest hydrophobicity will become localized in the membranes they encounter initially. Thus, an optimum hydrophobicity may be found in some test systems.

Figure 4. Parabolic correlation between log 1/C and MR.



Knowing this fact, we tried to find parabolic correlations and thus an excellent correlation is obtained between log1/C and MR, (Figure 4):

$$\log 1/C = - 0.0036\ MR^2 + 0.1601\ MR + 3.6405$$
$$R^2 = 0.9439 \quad SD=0.1378$$

For many QSAR studies many descriptors are needed. Addition of more then one descriptor would certainly improve the QSAR model.

Several multivariate correlations between the structural parameters mentioned above and log1/C are presented in Table 5, together with correlation coefficients (R) and standard deviations (SD).

In bivariate correlation analysis also, the correlations involving π, logP and σ are found to be good (Table 5, correlation No 1-13; R=0.91-0.96). Excellent correlation (0.9679) is obtained when in bivariate correlation logP and R were used. The correlation is expressed as:

$$\log 1/C = 0.7156\log P + 2.5765R + 3.9939$$
$$R=0.9679 \quad SD=0.1462$$

It is interesting to note that an excellent correlation is also obtained in tervariate correlation involving the same parameters (correlation No 14-18, Table 5). The correlation coefficients in all the cases were found to be approximately the same (0.97-0.98), and the standard deviation below 0.16.

Table 5. Regression parameters and the quality of correlation of log 1/C with σ, π, log P, MR, F, R and L, multivariate regressions for substituted 1,2,4-triazoles.

Correlation No	***Correlation parameters used*** [a]	***Slope Ai i=1-2***	***Intercept B***	***R***	***SD***
1	σ MR	A_1= 0.2668 A_2= 0.0528	4.1667	0.9389	0.2002
2	σ L	A_1= -0.2059 A_2= 0.3222	3.4557	0.9565	0.1697
3	π logP	A_1= -3.0467 A_2= 4.0843	1.6286	0.9130	0.2373
4	π MR	A_1= -0.2347 A_2= 0.0679	4.2092	0.9647	0.1542
5	π R	A_1= 0.6084 A_2= 2.8805	4.5146	0.9452	0.1899
6	π L	A_1= -0.0131 A_2= 0.3182	3.4419	0.9502	0.1812
7	logP MR	A_1= -0.2789 A_2= 0.0691	4.3958	0.9556	0.1714
8	logP R	A_1= 0.7156 A_2= 2.5765	3.9939	0.9679	0.1462
9	logP L	A_1= 0.0007 A_2= 003143	3.4492	0.9500	0.1815
10	MR R	A_1= 0.0495 A_2= 0.7650	4.2878	0.9599	0.1630
11	MR L	A_1= 0.0120 A_2= 0.2469	3.6068	0.9519	0.1780
12	F L	A_1= -0.4005 A_2= 0.3111	3.5676	0.9654	0.1517
13	R L	A_1= 0.1041 A_2= 0.3097	3.4743	0.9505	0.1807

Table 5. Cont.

Correlation No	*Correlation parameters used*[a]	*Slope Ai i=1-2*	*Intercept B*	*R*	*SD*
	σ	A_1= -0.6667			
14	π	A_2= -0.2226	3.3456	0.9736	0.1625
	L	A_3= 0.4036			
	π	A_1= -1.4854			
15	logP	A_2= 1.7219	3.0706	0.9866	0.1165
	MR	A_3= 0.0476			
	π	A_1= -1.2182			
16	logP	A_2= 2.0982	3.0139	0.9853	0.1217
	R	A_3= 1.8779			
	π	A_1= -1.7249			
17	MR	A_2= 0.1866	3.6820	0.9771	0.1513
	R	A_3= -5.1995			
	logP	A_1= 1.8755			
18	MR	A_2= -0.0812	3.5299	0.9728	0.1649
	R	A_3= 5.5237			

[a] x, y, z from the Eqn. 1; R - correlation coefficient; SD - standard deviation

After including the compounds with little structural modification (compounds **9** and **10**) the following correlations were obtained (Table 6):

Table 6. Regression parameters and the quality of correlation of log 1/C with σ, π, log P, MR, F, R and L; (log1/C = ΣAi x Ii + B).

Correlation No	*Correlation parameters used*[a]	*Slope Ai i=1-4*	*Intercept B*	*R*	*SD*
1.	I_H	A_1= 0.4783	3.8239	0.9034	0.2199
	MR	A_2= 0.0457			
2.	I_H	A_1= 0.6556	3.5334	0.9289	0.2124
	σ	A_2= 0.4197			
	MR	A_3= 0.0483			
3.	I_H	A_1= 0.6410	3.6390	0.9373	0.1998
	π	A_2= -0.2520			
	MR	A_3= 0.0629			
4.	I_H	A_1= 0.442	3.9815	0.9138	0.2328
	logP	A_2= -0.1849			
	MR	A_3= 0.0562			

Table 6. Cont.

Correlation No	***Correlation parameters used***[a]	***Slope Ai i=1-4***	***Intercept B***	***R***	***SD***
5.	I_H	A_1= 0.6674	3.5784	0.9387	0.2282
	σ	A_2 = 0.1454			
	π	A_3= -0.1976			
	MR	A_4= 0.0600			
6.	I_H	A_1= 0.6394	3.5760	0.9290	0.2449
	σ	A_2 = 0.3951			
	logP	A_3= -0.0300			
	MR	A_4= 0.0499			
7.	I_H	A_1= 1.5164	1.8039	0.9885	0.0909
	π	A_2 = -1.2034			
	logP	A_3= 1.3356			
	MR	A_4= 0.0525			

R - correlation coefficient; SD - standard deviation

As it can be seen from Table 6, an excellent correlation was obtained when π, logP and MR were used:

$$\log 1/C = 1.5164\ I_H - 1.2034\ \pi + 1.3356 \log P + 0.0525MR + 1.8039$$

where I_H is structural indicator parameter representing $–CH_2-$ group as 1 and CH_3-CH- group as 0. From the value of R (0.9885) can be seen that a relatively good model was chosen.

The last step was determination of the influence of heterocyclic ring by using the extend Free – Wilson equation. The following equation was obtained:

$$\log 1/C = 0.3094\ I_H + 0.3712\ I_{=CH-} - 0.2104 \log P + 4.6375$$
$$R = 0.7171 \quad SD = 0.3567$$

Extending the investigation system may lead to developing the better QSAR system when another heterocyclic nucleus, besides triazole, is included in the chosen substituted 1,2,4-triazoles.

3. Conclusions

Spurred by the need for new antimicrobial agents and the fact that many effective drugs, insecticides and fungicides possess heterocyclic systems in their structure, such as the triazole ring, we synthesized some new 1,2,4-triazole derivatives. Analysis of this limited set of substituted 1,2,4-triazole molecules allowed us to build a QSAR model of their antimicrobial activity against *B. subtilis* in which π, logP and σ are important factors. When we summarized the results derived from this limited set of triazole derivatives we came to the conclusion that the QSAR results don't really explain anything, but rather the QSAR equations just point to correlations. On the other hand, QSAR is a very important and routine method for many areas of chemistry. QSAR is best appreciated as a guide for our chemical intuition and QSAR models may guide us to what to synthesize next in our search for more effective solutions to our problems. This, in turn, will help medical as well as

agriculture chemist in their prediction of increasing activity and thus the synthesis of new triazoles exhibiting better activities then those reported in this paper.

4. Experimental

4.1 Microbiology

The filter paper disc method [13] was performed in Sabouraud dextrose broth and Mueller Hinton broth. These agar media were inoculated with 24 h liquid cultures containing 10^7 microorganisms/mL (0.5 mL). Filter paper discs (5 mm diameter) saturated with each compound solution (1 mg/mL; 5 mg/mL and 10 mg/mL in DMSO) were placed on the indicated agar mediums. The incubation time was 24 h at 37 ºC for bacterial and 48 h at 30 ºC for *Candida sp*. Discs with DMSO were used as control. The diameter of zone inhibition (mm) was measured. The tests were repeated 3 times to confirm the findings.

4.2 QSAR analysis

The MVA (multi variable analysis) approach in QSAR analysis has been most widely and effectively used for theoretical drug design due to various physicochemical (electronic, steric and hydrophobic) parameters and structural indicator parameters used together (Hansch and Free Wilson approaches) [1,2].

The assumption can be formulated as given in Eqn. 1, (Hansch approach):

$$log1/C = A_1 x + A_2 y + A_3 z + B \qquad Eqn.\ 1$$

where *x*, *y* and *z* are molecular properties, and log1/C is the desired biological activities. From the values of linear slopes A_1, A_2, A_3 we can see the correlation of the particular molecular properties with the activity of the investigated compounds.

Applying the same chosen descriptors in Free Wilson analysis (Eqn. 2) the activity contributions of either methyl- or substituted heterocyclic ring systems were determined from the correlation equation:

$$log1/C = \Sigma a_i I_i + \Sigma b_i x_i + B \qquad Eqn.\ 2$$

where I_i is the structural indicator parameter; x_i and log1/C had the same meaning as in Eqn.1.

The variables used as descriptors in the analysis are electronic, steric and structural parameters (Tables 3 and 7). Physicochemical parameters taken into consideration in QSAR study are σ electronic parameter of substituents, π hydrophobic parameter, F (field effect) as electronic influences, Verloop's STERIMOL parameter L for the steric interactions of the substituents R_2. L is defined as the length of a substituent along the axis of its substitution to the parent skeleton. Electronic effect of the substituents, expressed in term of F, is found to be important in determining the activity, as it is predictive in electrophilic reactions of bimolecules. The classical Hammett σ parameters and MR value were used (Tables 3 and 7). For each compound the partition coefficient logP has been calculated [12] (Table 3).

Table 7*:* Physicochemical parameters of the triazole derivatives studied

R	σ^a	π^a	***F*** [a]	***R*** [a]	***L*** [a]
p-$COOC_2H_5$	0.45	0.51	0.33	0.15	5.96
p-COOH	0.45	-0.32	0.33	0.15	3.91
o-COOH	1.2	-0.32	0.33	0.15	3.91
p-Cl	0.23	0.71	0.41	-0.15	3.52
p-Br	0.23	0.86	0.44	-0.17	3.83
p-CH_3	-0.17	0.56	-0.04	-0.13	3.00
p-C_6H_5	-0.01	1.96	0.08	-0.08	6.28
p-NO_2	0.78	-0.28	0.67	0.16	3.44

[a] Ref. [1]

Applying the Free Wilson analysis, in first step, the structural variable indicator I_H expresses the replacement of hydrogen atom by the methyl group in the aminomethyl unit. I_H is defined as 1 for the N^1-aryl/heteroarylaminomethyl-1,2,4-triazoles (**1**-**8**, **11**-**16**), and 0 for N^1-aryl/heteroarylaminoethyl-1,2,4-triazole derivatives (**9**,**10**,**17**,**18**). In second step, the other indicator $I_{=CH-}$ is defined as 1 for compounds with =CH- in the six membered ring (**1**-**10**), and 0 for compound with –N= group in the six membered ring (**11**-**18**) (Table 8).

Table 8. Matrix for Free Wilson approach.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
$\mathbf{I_H}$	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1
$\mathbf{I_{=CH-}}$	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0

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