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QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS OF SOME N^1-ARYL/HETEROARYLAMINOMETHYL/ETHYL-1,2,4-TRIAZOLES

PART III: ANTIMICROBIAL ACTIVITY AGAINST *SALMONELLA ENTERITIDIS*

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Quantitative structure activity relationships (QSAR) analysis of a series of previously synthesized N^1-arylaminomethyl/ethyl-1,2,4-triazole and N^1-heteroarylaminomethyl/ethyl-1,2,4-triazole derivatives tested for growth inhibitory activity against *Salmonella enteritidis,* 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 and aromatic/heteroaromatic ring was determined from the correlation equation. An excellent parabolic correlation was obtained between log1/C and *F* (regression coefficient *R* is 0.9800). In a bivariate correlation analysis, the correlations involving σ, MR and *F* were found to be good (R = 0.90 – 0.99). An excellent correlation was also obtained in tervariate correlation involving the same parameters (R = 0.995 – 0.999), with standard deviations below 0.06.

Key words: QSAR/QSPR; N^1-heteroarylaminomethyl/ethyl-1,2,4-triazole; physico-chemical parameters

КОРЕЛАЦИЈАТА СТРУКТУРА–РЕАКТИВНОСТ НА НЕКОИ N^1-АРИЛ/ХЕТЕРОАРИЛАМИНОМЕТИЛ/ЕТИЛ-1,2,4-ТРИАЗОЛИ

III ДЕЛ: АНТИМИКРОБНА АКТИВНОСТ ВО ОДНОС НА *SALMONELLA ENTERITIDIS*

Применет е методот на воспоставување корелација помеѓу структурата и активноста (QSAR) на серија претходно синтетизирани N^1-ариламинометил/етил-1,2,4-триазоли и N^1-хетероариламинометил/етил-1,2,4-триазоли, со користење на резултатите добиени од антимикробните испитувања во однос на *Salmonella enteritidis*. Со примена на методите на Hansch и Free Wilson се утврдени уделите на влијанијата на аминометил/аминоетилната единица и на ароматичниот/хетероароматичниот прстен во молекулата на биолошката активност на испитуваните соединенија, со интерпретација на добиените корелациони равенки. Одлична е параболичната зависност помеѓу log 1/*C* и *F* (коефициентот на корелација е 0,9800). Задоволителни се корелациите добиени од зависноста log1/*C* и избрани два (R = 0,90 – 0,99) и/или три дескриптори (R = 0,995 – 0,999) и стандардна девијација под 0,06.

Клучни зборови: QSAR; N^1-арил/хетероариламинометил/етил-1,2,4-триазоли; физичко-хемиски параметри

INTRODUCTION

Our society is faced with challenges that can 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; hence, it is necessary to develop predictive tools for molecular properties and environmental setting. Quantitative structure

activity relationships (QSAR) and quantitative structure properties relationships (QSPR) play a central role in this effort, and those 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 a structure with the desired properties. It is then possible to select the most promising compounds for synthesis and testing 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 [4–8].

Consequently, spurred by the need of new antimicrobial agents and the fact that many new effective antimicrobial drugs possess heterocyclic rings in their structure, such as the 1,2,4-triazole ring, we synthesized some new 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* during the last few years [9–13]. The antibacterial activity against *Salmonella enteritidis* of synthesized N^1-aryl/heteroarylaminomethyl/ethyl-1,2,4-triazole derivatives were used in this QSAR analysis.

EXPERIMENTAL

Materials

All the N^1-aryl/heteroarylaminomethyl/ethyl-1,2,4-triazole derivatives (1–18), (Tables 1 and 2; Fig. 1 and 2), used in this study were previously synthesized and reported elsewhere [9, 10].

Table 1

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

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

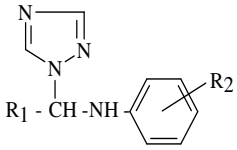
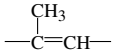


Fig.1. N^1-arylaminomethyl/ethyl-1,2,4-triazole derivatives (1–10)

Table 2

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

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

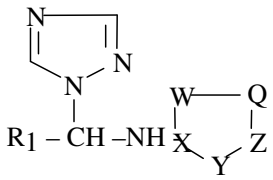


Fig. 2. N[1]-heteroarylaminomethyl/ethyl-1,2,4-triazole derivatives (11–18)

Microbiology

The filter paper disc method [14] was performed in Sabouraud dextrose broth and Mueller Hinton broth. These agar media were inoculated with 0.5 ml of the 24 h liquid cultures containing 10^7 microorganisms/ml. Filter paper discs (5 mm diameter) saturated with each compound solution (1 mg/ml; 5 mg/ml and 10 mg/ml dimethyl sulfoxide – DMSO) were placed on the indicated agar mediums. The incubation time was 24 h at 37°C for bacterial and 48 h at 30°C 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.

QSAR analysis

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

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

$$\log 1/C = A_1 x + A_2 y + A_3 z + B \qquad (1)$$

where *x, y* and *z* are molecular properties, and log1/*C* is desired biological activities. From the values of the 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 Willson analysis (Eq. 2) the activity contributions of either methyl- or substituted heterocyclic ring systems were determined:

$$\log 1/C = \Sigma a_i I_i + \Sigma b_i x_i + B \qquad (2)$$

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

The variables used as descriptors in the analysis are electronic, steric and structural parameters (Tables 3 and 4). 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 (Table 3). For each compound the partition coefficient log*P* has been calculated [15] (Table 4).

Table 3

Physicochemical parameters of triazole derivatives studied

R	σ^a	π^a	F^a	R^a	L^a	MR^a
p-$COOC_2H_5$	0.45	0.51	0.33	0.15	5.96	*17.47*
p–COOH	0.45	–0.32	0.33	0.15	3.91	*6.93*
o–COOH	1.2	–0.32	0.33	0.15	3.91	*6.93*
p–Cl	0.23	0.71	0.41	–0.15	3.52	*6.03*
p–Br	0.23	0.86	0.44	–0.17	3.83	*8.88*
p–CH_3	–0.17	0.56	–0.04	–0.13	3.00	*5.65*
p–C_6H_5	–0.01	1.96	0.08	–0.08	6.28	*25.36*
p–NO_2	0.78	–0.28	0.67	0.16	3.44	*0.67*

[a] Taken from Ref. [1]

Table 4

Calculated log P values for compounds (1–16)

Compounds	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(16)
log P^a	1.2981	0.4579	1.4690	1.2210	1.4665	1.1238	2.3405	–1.6900	1.7157	1.3895	0.0301	0.5774	0.5774	–2.1800

[a] Taken form Ref [15]

Applying the Free Willson analysis, in the 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) (Tab. 5).

Table 5

Matrix for Free Willson approach

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

RESULTS AND DISCUSSION

In our work, the chosen model is based on the *in vitro* antimicrobial activity of certain N^1-aryl-aminomethyl/ethyl-1,2,4-triazoles and N^1-heteroarylaminomethyl/ethyl-1,2,4-triazoles derivatives (1–18) (Tables 1 and 2; Figs 1 and 2), against *Salmonella enteritidis,* where *C* is the minimum inhibition concentration (MIC) value expressed in molar concentration units (Table 6).

Table 6

Experimental obtained MIC values, calculated log 1/C and log P

Compounds No:	MIC[a]	log 1/ *C*
(1)	4.061×10^{-6}	5.391
(2)	4.583×10^{-6}	5.339
(3)	–	–
(4)	4.793×10^{-6}	5.319
(5)	3.951×10^{-6}	5.403
(6)	2.656×10^{-5}	4.576
(7)	1.998×10^{-5}	4.699
(8)	3.842×10^{-5}	4.415
(9)	1.921×10^{-5}	4.716
(10)	–	–
(11)	5.708×10^{-6}	5.244
(12)	5.399×10^{-6}	5.267
(13)	5.399×10^{-5}	4.267
(16)	6.055×10^{-6}	5.218

[a]Minimum inhibition concentration expressed in molar concentration.

The results of the antimicrobial investigation indicate 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 *Salmonella enteritidis*, expressed as MIC, are given in Table 6.

After applying the filter paper disc method [14], it was determined that compounds (3), (10) and (15) do not inhibit the growth of the chosen microorganism [10]. From the obtained data, first the values for MIC were calculated and then the log 1/*C* values were obtained (Tab. 6).

In our work, attempts were made for making correlation between selected physicochemical properties and experimental values for antimicrobial activities against *Salmonella enteritidis*, in three ways:

– applying general Hansch equation for structurally identical compounds (1–8);

– using Free and Wilson approach which includes derivatives with some structural changes (aminomethyl unit has been replaced with aminoethyl group), compounds (1–10);

– extend Free–Wilson equation, for determination of the influence of the heterocyclic ring, substituted on the amino group, compounds (1–18).

When the data in Tables 3, 4 and 6 were submitted to linear regression (Fig. 3), the resulting QSAR equation is:

$$\log 1/C = 1.4148\ \sigma + 4.8501 \qquad (3)$$

$$R = 0.8919 \qquad SD = 0.1980$$

where *R* is correlation coefficient, SD is standard deviation.

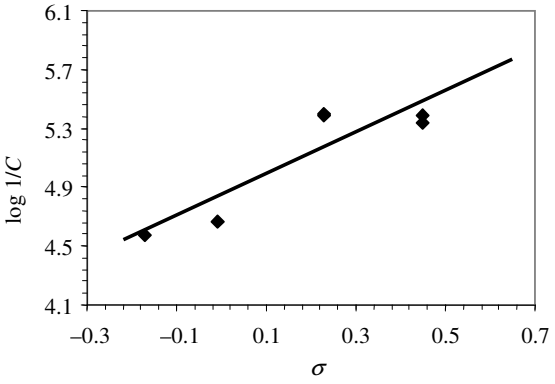


Fig. 3. Linear correlation between log 1/*C* and σ

The data for the chosen compounds were reasonably correlated with a regression coefficient of 0.8919, indicating a relatively good fit (Eq. 3).

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

Attempt was also made for making a parabolic correlation. Parabolic relationships between biological response (log1/*C*) and log*P* term can be explained by the fact that many membranes must be traversed so that compounds can 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.

Knowing this fact, we tried to find a parabolic correlation, and indeed an excellent correlation is obtained between log 1/*C* and *F* (Fig. 4; Eq. 4):

$$\log 1/C = 0.8864\ F^2 + 1.6131\ F + 4.6251 \quad (4)$$

$$R^2 = 0.9605 \qquad \text{SD} = 0.1084$$

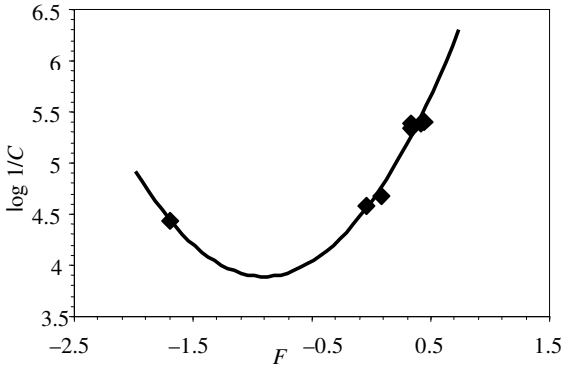


Fig. 4. Parabolic correlation between log 1/*C* and *F*

For many QSAR study numerous 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 7, together with correlation coefficients and standard deviations.

In a bivariate correlation analysis, the correlations involving σ, *MR* and *F* are found to be good (Tab. 7, correlation No 1–9; *R* = 0.90–0.99). Excellent correlation (0.9902) is obtained when, in bivariate correlation, σ and *R* were used. The correlation is expressed as:

$$\log 1/C = 2.0859\ \sigma - 1.5945\ R + 4.6570 \quad (5)$$

$$R = 0.9902 \qquad \text{SD} = 0.0708$$

Table 7

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 A_i $i = 1 - 2$	Intercept *B*	*R*	SD
1	σ	A_1= 1.3903	4.9942	0.9239	0.1944
	MR	A_2= –0.0119			
2	σ	A_1= 0.4835	4.6509	0.9899	0.0719
	F	A_2= 1.4798			
3	σ	A_1= 2.0859	4.6570	0.9902	0.0708
	R	A_2= –1.5945			
4	σ	A_1= 1.4990	5.1629	0.9269	0.1906
	L	A_2= –0.0746			
5	π	A_1= –0.1022	4.7234	0.9898	0.0719
	F	A_2= 1.8498			
6	log*P*	A_1= –0.1099	4.7883	0.9868	0.0819
	F	A_2= 1.8774			
7	*MR*	A_1= –0.0029	4.6577	0.9750	0.1128
	F	A_2= 1.9509			
8	*F*	A_1= 1.9268	4.6489	0.9898	0.0724
	R	A_2= 0.4788			
9	*F*	A_1= 1.9796	4.6244	0.9734	0.1163
	L	A_2=–0.0017			
10	σ	A_1= 2.0184	4.7370	0.9953	0.0603
	MR	A_2= –0.0053			
	R	A_3= –1.4598			
11	π	A_1= –0.5474	4.3516	0.9962	0.054
	log*P*	A_2= 0.5362			
	F	A_3= 1.7829			
12	π	A_1= –0.1428	4.5938	0.9960	0.0554
	F	A_2= 1.8137			
	L	A_3= 0.0380			
13	*MR*	A_1= –0.0524	4.0613	0.9997	0.0145
	F	A_2= 1.5595			
	L	A_3= 0.2985			

[a] *x, y, z* from the Eq. 1; *R* – correlation coefficient; SD – standard deviation

It is interesting to note that an excellent correlation is also obtained in tervariate correlation involving the same parameters (correlation No 10–13, Tab. 7). The correlation coefficients in all the cases were found to be approximately the same (0.995–0.999), with standard deviations were below 0.06.

After including the compounds with little structural modification (compounds 9 and 10), the following correlations were obtained (Tab. 8).

Table 8

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 A_i $i = 1 – 4$	Intercept B	R	SD
1	I_H	A_1= 0.7704	4.0790	0.9099	0.1989
	σ	A_2= 1.4148			
2	I_H	A_1= 0.7707	4.078	0.9099	0.2297
	σ	A_2= 1.4170			
	π	A_3= 0.0014			
3	I_H	A_1= 0.7599	4.1137	0.9101	0.2295
	σ	A_2= 1.3970			
	log P	A_3=–0.0151			
4	I_H	A_1= 0.6956	4.2984	0.9363	0.1944
	σ	A_2= 1.3903			
	MR	A_3=–0.0119			
5	I_H	A_1= 0.1939	5.3887	0.9259	0.2561
	σ	A_2 = 1.7209			
	π	A_3= 0.9909			
	log P	A_4= –1.1378			
6	I_H	A_1= 0.6178	4.2629	0.9947	0.0698
	σ	A_2 = 2.0398			
	π	A_3= 0.4257			
	MR	A_4= –0.0390			
7	I_H	A_1= 0.8816	3.6382	0.9991	0.0291
	σ	A_2 = 1.9836			
	π	A_3= 0.5628			
	MR	A_4= –0.0447			

[a] *x, y, z* from the Eq. 1; R – correlation coefficient; SD – standard deviation

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

$$\log 1/C = 0.3094\, I_H + 0.3712\, I_{=CH-} - 0.2104 \log P + 4.6375 \quad (6)$$

$$R = 0.4498 \qquad SD = 0.4455$$

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

CONCLUSIONS

Spurred by the need of 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 *S. enteritidis*, in which π, log$P$ and σ are important factors.

When we summarized all the results of this limited set of triazole derivatives, we came to the following conclusion: the QSAR results don't really explain anything; 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. QSAR models 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 agricultural chemists in their prediction of increased activity and thus enable the synthesis of new triazoles exhibiting better activities then those reported in this paper.

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