Eur. J. Med. Chem. 34 (1999) 753–758

Short communication

QSAR study on antibacterial and antifungal activities of some 3,4-disubstituted-1,2,4-oxa(thia)-diazole-5(4 H)-ones(thiones) using physicochemical, quantumchemical and structural parameters

Ömer Geban[a], Hamide Ertepinar[a], Mine Yurtsever[b], Seçkin Özden[c]*, Fatma Gümüs[d]

[a]*Middle East Technical University, Faculty of Education, Department of Science Education, 06531 Ankara, Turkey*
[b]*Istanbul Technical University, Faculty of Arts and Sciences, Department of Chemistry, Istanbul, Turkey*
[c]*Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Tandoğan- Ankara, Turkey*
[d]*Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Ankara, Turkey*

(Received 19 October 1998; revised 3 March 1999; accepted 11 March 1999)

Abstract – This work demonstrated the quantitative structure-activity relationships of 3,4-disubstituted-1,2,4-oxa(thia)-diazole-5(4 H)-ones (thiones) using quantum chemical parameter R(*I*), hydrophobicity descriptor and structural parameters. Semiempirical molecular orbital calculations were used to determine the quantum chemical parameter R(*I*), which is the electron density of HOMO at the sulfur and oxygen in position 1 of the compounds investigated, divided by the orbital energy of HOMO. It was shown that the electron density of HOMO at the sulfur and oxygen of the molecules was strongly related to the biological activities of these molecules. The results obtained from the QSAR application indicated that there was a parabolic dependence between the biological activities and the R(*I*) index. The structural factor I_Y which shows the presence of a sulfur atom in position 1 was the dominant predictor for the antibacterial and antifungal activities. On the other hand, the other structural variable I_X which shows the presence of a sulfur atom double bonded to the C atom in position 5 caused a decrease, but the hydrophobicity of the whole molecule (Σ) caused an increase in activity. © 1999 Éditions scientifiques et médicales Elsevier SAS

3,4-disubstituted-1,2,4-oxa(thia)-diazole-5(4 H)-ones(thiones) / QSAR / antibacterial activity / antifungal activity / quantum chemical calculations

1. Introduction

A series of compounds having the 3,4-disubstituted-1,2,4-oxa(thia)-diazole-5-(4H)-ones(thiones) structure (*figure 1*) was found to show antibacterial and antifungal activities [1]. These compounds were tested in vitro for their ability to inhibit the growth of representative gram-positive and gram-negative bacteria and various fungal species.

In order to identify the substituent effects to the chemical reactivity and the biological activity, we have examined the QSAR of a series of these compounds using the quantum chemical index R(*I*), physicochemical and structural parameters.

Recently, much research which concerns the quantum chemical parameters in QSAR studies have been reported [2–6]. Theoretical calculations have been used for the description of the mechanism of interactions at the molecular level. The electrophilic and nucleophilic superdelocalizability of nitrogen [6], sulfur and oxygen atom [5] and electron densities on the sulfur and oxygen atoms [4] in the investigated structures have been used as

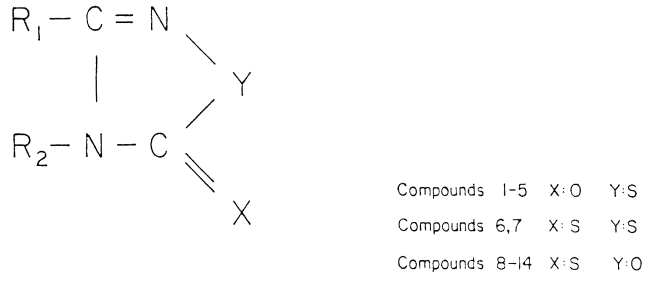

Figure 1. Structures of compounds **1**–**14.**

*Correspondence and reprints

Table I. Biological activities of the compounds **1**–**14**.

Compound	R_1	R_2	S.a.[a]	B.s.[b]	P.a.[c]	C.a.[d]	C.p.[e]	C.p.[f]	C.n.[g]
1	Ph	Me	3.58	3.58	3.58	3.89	3.89	3.89	4.19
2	2-Pyr	Me	3.89	3.89	3.89	4.19	4.19	4.19	4.19
3	2-Pyr	Ph	4.01	4.01	4.01	4.31	4.31	4.31	4.31
4	2-Pyr	Et	3.92	4.22	4.22	3.92	4.22	4.22	4.22
5	4-Pyr	p-tolyl	3.73	3.73	3.73	4.03	4.03	4.03	4.03
6	Ph	H	3.89	3.89	3.89	3.89	4.19	3.89	4.19
7	Ph	p-tolyl	4.06	4.06	4.06	4.06	4.36	4.06	4.36
8	2-Pyr	Et	2.72	2.72	3.02	3.02	3.02	3.02	3.32
9	2-Pyr	Me	2.99	2.99	2.99	3.29	3.29	3.29	3.29
10	2-Pyr	n-pr	3.05	3.05	3.05	3.35	3.35	3.35	3.65
11	Me	Ph	2.98	3.28	2.98	3.28	3.28	3.28	3.28
12	4-Pyr	Et	3.32	3.32	3.32	3.02	3.02	3.02	3.02
13	4-Pyr	Me	2.99	2.99	2.69	2.99	3.29	3.29	3.29
14	2-Pyr	Ph	3.11	3.11	3.11	3.41	3.41	3.41	3.41

[a]*S. aureus,* [b]*B. subtilis,* [c]*P. aeruginosa,* [d]*C. albicans,* [e]*C. pseudotropicalis,* [f]*C. parapsilosis,* [g]*C. neoformans.*

the independent parameters in QSAR analysis. The results obtained from these researches showed that the main type of interaction at the binding site should be via interaction of the lone pairs of heteroatoms in the compounds which were investigated with any binding site(s).

2. Chemistry

2.1. Chemicals and biological activity

The compounds investigated in this work were synthesized previously [7–9]. The antibacterial activity against *Staphylococcus aureus, Bacillus substilis* and *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans, Candida pseudotropicalis, Candida parapsilosis and Crytococcus neoformans* were determined [1] using the tube dilution method, and given as minimum inhibitory concentration (MIC, g/mL). The potency was defined as log1/C in the QSAR analysis, where C is the molar MIC value of the compound and is used as the dependent variable in the QSAR study. The log1/C values are given in *table I*.

2.2. Molecular orbital calculations

The AM1 [10] molecular orbital calculations were carried out using the MOPAC program [11] running on a Silicon Graphics Work station (IRIS 4D, INDIGO, POWER INDIGO 2). Initially the geometries of the molecules were fully optimized. After optimization, ISF calculations with VECTORS option were carried out to print all eigen values, eigen vectors, and HOMO energies *(table II)*.

2.3. Quantitative structure-activity relationships (QSAR)

QSAR of 3,4-disubstituted-1,2,4-oxa(thia)-diazole-5(4 H)-ones(thiones) was investigated using the quantum-chemical parameter R(*I*), physicochemical parameter (π) and structural parameters.

The electron density of HOMO at active sites of the molecules (position 1) are strongly related to the antibacterial and antifungal activities of the molecules. These active sites are important in such a way that they bind to some specific SH enzymes to achieve the role of inhibition. Previously Nakayama et al. [4] introduced an index R(*I*) which shows the electron density of HOMO at atom i which is one of the active sites (in the molecule) playing a role in the possible chemical reactions. It was defined as

$$R(I) = (fr(i)/-E_{HOMO}) \times 10^2$$

where fr(i) is the frontier electron density of HOMO at atom i and $-E_{HOMO}$ is the energy of HOMO in eV measured from the zero level, f(i) is the measure of the delocalization of electron density of HOMO on atom i and $-E_{HOMO}$ is the energy of the highest occupied (most energetic) molecular orbital. These two parameters are a measure of the relative reactivity of HOMO at atom i within a single molecule. Then, the ratio of fr(i) and $-E_{HOMO}$ at atom i can be related to the biological reactivity of a series of molecules concerned.

Oxygen and sulfur atoms embedded in the five membered ring of the concerned structure were thought to be the effective part of these compounds. Then, to investigate the contribution of this index to the biological activities of the examined molecules, the R(*I*) values at the S and O atom in position 1 were calculated using the MOPAC program and the values are listed in *table II*.

Table II. QSAR parameters examined in this study for the compounds **1**–**14**.

	X	Y	E_{HOMO}(eV)	R(*I*)	$\sum\pi$	I_X	I_Y
1	O	S	–9.105	5.37	2.52	0	1
2	O	S	–9.559	4.32	1.06	0	1
3	O	S	–9.310	1.03	2.46	0	1
4	O	S	–9.560	4.37	1.52	0	1
5	O	S	–9.410	0.83	3.01	0	1
6	S	S	–8.961	3.55	1.96	1	1
7	S	S	–8.824	3.55	4.65	1	1
8	S	O	–9.067	1.04	1.52	1	0
9	S	O	–9.008	1.03	1.06	1	0
10	S	O	–9.059	1.03	2.05	1	0
11	S	O	–9.017	0.81	2.52	1	0
12	S	O	–9.274	1.06	1.34	1	0
13	S	O	–9.285	1.05	0.88	1	0
14	S	O	–8.984	0.93	2.46	1	0

Concerning physicochemical parameters, the hydrophobicity of the compounds (π) were used in the QSAR analysis. π values of the substituents were taken from the tables given by Hansch and Leo [12] and Σ was used for the hydrophobicity of the whole molecule. The structural parameter (I_Y) expresses the presence of S and O atoms in position 1. I_Y is defined as 1 in the case of the presence of S in the position 1 and 0 for the O in the same position. The other structural variable (I_X) represents the atom which is double bonded to the C atom in position 5. Similarly I_X has a value of 1 for the S atom and 0 for the O atom. *Table II* shows the parameters used in this study.

3. Results and discussion

3.1. R(I) index and the biological activities of 3-4-disubstituted 1,2,4-oxa(thia)-diazole-5(4 H)-ones(thiones)

Antibacterial and fungicidal activities of these compounds are given in *table I*. From *table I* it can be seen that molecules that contain O in position 1 (**8–14**) have lower activities than the molecules which have S in position 1 (**1–7**). Similarly the R(*I*) index is much lower for the O containing molecules than the others. Accordingly there is a correlation between R(*I*) and biological activities measured. Namely, sulfur containing molecules in position 1 have more electron density on the sulfur atom under consideration coming from HOMO, which makes that region more susceptible to chemical reactions, especially to the ones in which binding to another atom or electron transfer reactions are considered.

3.2. QSAR

Correlation and regression analyses of the QSAR study were done on a microcomputer using the SPSS/PC [13]. In the equations, the figures in parantheses are the standard errors of the regression coefficients, *n* is the number of compounds, r is the multiple correlation coefficient, s is the standard error of estimate, and F is the ratio of regression and residual. The level of significance accepted in all of the analyses was 0.05.

The best equation(s) was selected among other equations by considering the various statistical criteria [14–17]. All possible combinations of parameters were considered, except that square terms were only allowed in equations containing the corresponding linear terms. This method provided a total of 22 possible equations for each activity. The results showed that there were two best equations including the same terms for each activity. The first best fitted equations included R(*I*), $R(I)^2$, Σ, and I_X for the activities against *S. aureus, B. subtilis, P. aeruginosa, C. albicans, C. pseudotropicoli, C. parapsilosis,* and *C. neoformans*. The second best fitted equation for each activity included only the structural parameter I_Y. *Table III* shows the best equations for the antibacterial and antifungal activities of the examined structures. In these equations, *n* represents the number of compounds analysed, r, the multiple correlation coefficient, s, the standard deviation, and F, the ratio of regression and residual. The figure in parantheses is the 95% confidence interval.

The equations including R(I), $R(I)^2$, Σ, and I_X together for the activities against *S. aureus, B. subtilis, P. aeruginosa, C. albicans, C. pseudotropicoli, C. parapsilosis,* and *C. neoformans* were all significant because the overall F statistics for these equations were statistically significant, and they have high r values (about 0.90). Also, the individual F statistics for the coefficients of R(I), $R(I)^2$, Σ, and I_X in these equations were significant at $P < 0.05$ *(table IV)*.

Table III. The best equations.

Number	Equation	*n*	r	s	F	p
	The best equations against *S. aureus*					
1	log1/C = 0.85 (± 0.20) I_Y + 3.02	14	0.94	0.17	84.61	0.0000
2	log1/C = 1.05 (± 0.46) R(*I*) – 0.18(± 0.085) R(*I*)^2 + 0.14 (± 0.11)$\sum\pi$ – 0.66 (± 0.28) I_X + 2.59	14	0.95	0.17	20.19	0.0002
	The best equations against *B. subtilis*					
3	log1/C = 0.85 (± 0.24) I_Y + 3.06	14	0.91	0.20	58.39	0.0000
4	log1/C = 1.05 (± 0.51) R(*I*) – 0.17 (± 0.09) R(*I*)^2 + 0.14 (± 0.12)$\sum\pi$ – 0.68 (± 0.31) I_X + 2.65	14	0.94	0.19	17.49	0.0003
	The best equations against *P. aeruginosa*					
5	log1/C = 0.89 (± 0.23) I_Y + 3.02	14	0.92	0.20	68.94	0.0000
6	log1/C = 1.13 (± 0.44) R(*I*) – 0.19 (± 0.079) R(*I*)^2+ 0.14 (± 0.11)$\sum\pi$ – 0.72 (± 0.27) I_X + 2.57	14	0.96	0.17	26.65	0.0001
	The best equations against *C. albicans*					
7	log1/C = 0.85 (± 0.19) I_Y + 3.19	14	0.94	0.17	87.35	0.0000
8	log1/C = 0.75 (± 0.45) R(*I*) – 0.13 (± 0.083) R(*I*)^2 + 0.17 (± 0.11)$\sum\pi$ – 0.76 (± 0.28) I_X + 3.06	14	0.95	0.17	21.16	0.0001
	The best equations against *C. pseudotropicalis*					
9	log1/C = 0.93 (± 0.18) I_Y + 3.24	14	0.95	0.16	121.29	0.0000
10	log1/C = 1.07 (± 0.44) R(*I*) – 0.18 (± 0.08) R(*I*)^2 + 0.18 (± 0.11)$\sum\pi$ – 0.71 (± 0.27) I_X + 2.79	14	0.96	0.17	27.45	0.0000
	The best equations against *C. parapsilosis*					
11	log1/C = 0.85 (± 0.19) I_Y + 3.24	14	0.94	0.16	99.18	0.0000
12	log1/C = 0.82 (± 0.38) R(*I*) – 0.14 (± 0.069) R(*I*)^2 + 0.13 (± 0.09)$\sum\pi$ – 0.79 (± 0.23) I_X + 3.14	14	0.97	0.15	30.99	0.0000
	The best equations against *C. neoformans*					
13	log1/C = 0.89 (± 0.18) I_Y + 3.32	14	0.95	0.15	120.33	0.0000
14	log1/C = 0.79 (± 0.46) R(*I*) – 0.12 (± 0.085) R(*I*)^2 + 0.18 (± 0.11)$\sum\pi$ – 0.61 (± 0.28) I_X + 2.99	14	0.95	0.17	21.67	0.0001

The predictor variables R(I), R(I)^2, Σ, and I_X accounted for a significant portion of the variance in the activities. Also, each predictor contributed a significant proportion of the additional variance in the presence of the other variables for each activity. The structural parameter I_X induced some influences on the activity. But it showed that S, which is double bounded to the C atom in position 5, caused a decrease in the activity. R(I) and its square produced an additive contribution to the activity. There was a parabolic dependence between the biological activities and the R(I) index. The parabolic relationship of the activity against *S. aureus* on the reactivity of HOMO on the sulfur and oxygen of 3,4 disubstituted 1,2,4-oxa(thia)-diazole-5(4 H)-one(thione) derivatives suggests that log1/C initially increases as the reactivity of HOMO increases up to an ideal R(I) value, and then it decreases after this point. Σ is the summation of the hydrophobic parameter of the substituents R_1 and R_2 in the observed structure and its positive coefficient suggested that higher hydrophobicity was favourable for the activity.

An examination of the intervariable correlations involving all predictor variables in *table V* demonstrates the relatively large correlation between R(I) and I_Y (r = 0.71) (colinearity).

When high colinearity exists, the regression analyses using the given set of independent variables can not be

Table V. Correlation matrix of all predictor variables used in the equations.

	R(*I*)	$\sum\pi$	I_Y	I_X
R(*I*)	1.000			
$\sum\pi$	0.121	1.000		
I_Y	0.712	0.399	1.000	
I_X	–0.483	–0.033	–0.745	1.000

Table IV. The *t* statistics ($P < 0.05$) for the coefficients of variables in the best equations 2, 4, 6, 8, 10, 12, 14.

	t	P
Equation 2		
R(*I*)	5.128	0.0006
$R(I)^2$	4.688	0.0011
$\sum\pi$	2.850	0.0191
I_X	5.264	0.0005
Equation 4		
R(*I*)	4.652	0.0012
$R(I)^2$	4.228	0.0022
$\sum\pi$	2.539	0.0317
I_X	4.921	0.0008
Equation 6		
R(*I*)	5.863	0.0002
$R(I)^2$	5.333	0.0005
$\sum\pi$	2.974	0.0156
I_X	6.085	0.0002
Equation 8		
R(*I*)	3.720	0.0048
$R(I)^2$	3.454	0.0072
$\sum\pi$	3.523	0.0065
I_X	6.223	0.0002
Equation 10		
R(*I*)	5.556	0.0004
$R(I)^2$	5.043	0.0007
$\sum\pi$	3.695	0.0050
I_X	6.062	0.0002
Equation 12		
R(*I*)	4.925	0.0008
$R(I)^2$	4.547	0.0014
$\sum\pi$	3.139	0.0119
I_X	7.757	0.0000
Equation 14		
R(*I*)	3.863	0.0038
$R(I)^2$	3.300	0.0092
$\sum\pi$	3.530	0.0064
I_X	4.880	0.0009

performed effectively [14, 18]. Also, the correlations between the biological activities and predictor variables demonstrates the highest correlation between the biological activities and structural parameter I_Y (r values about 0.90). For these reasons, the variable I_Y was entered into the correlation equation separately, and the second best equations were obtained *(table III)*. F-test values for each activity were significant. It showed the presence of the sulfur atom in position 1 was a dominant predictor for the activities against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *C. albicans*, *C. pseudotropicoli*, *C. parapsilosis*, and *C. neoformans.*

Table VI. The calculated r^2_{PRESS} and s_{PRESS}.

	r^2_{PRESS}	s_{PRESS}
Equation 1	0.831	0.201
Equation 2	0.472	0.409
Equation 3	0.768	0.242
Equation 4	0.756	0.286
Equation 5	0.798	0.234
Equation 6	0.751	0.299
Equation 7	0.836	0.198
Equation 8	0.578	0.366
Equation 9	0.877	0.185
Equation 10	0.637	0.367
Equation 11	0.853	0.186
Equation 12	0.806	0.246
Equation 13	0.877	0.177
Equation 14	0.383	0.457

In order to judge the validity of the predictive power of the QSAR, a cross-validation method was applied to the original data set for equations 1–14 and the resulting PRESS (predicted residual sum of squares) for each equation was calculated according the following equation [14, 19].

$$PRESS = \sum_i [(y_i - \hat{y}_i)^2 / (1 - h_{ii})^2]$$

here y_i and $\hat{y}_i$ are the response (activity) values of observation i (i = 1,2,..., n), observed and calculated by the best equation, respectively. The diagonal elements of that matrix are denoted by h_{ii} in the equation, and calculated by the SPSS computer program. The calculated overall cross-validated r^2 and standard deviation values for each equation are given in *table VI*.

The cross validation technique was used to check the validation of these equations, because this technique seems to substantially reduce the probability of chance correlation relative to multiple regression. In the present study, cross validation did not confirm some of the four-term equations. The cross-validated r^2 values had a maximum at one-term equations. Cross validation results using I_Y as the only independent variable were much better than those including four parameters R (I), $R(I)^2$, Σ, and I_X. One parameter equations were very stable, leading to cross-validated values in the range between 0.768–0.877, whereas the four-parameter equations gave cross-validated values between 0.383–0.806. This showed that one-term equations indicated higher predictive ability, as shown by cross validation. On the other hand, the greater the number of variables tested, the greater role chance will play in the observed correlation [20]. With an increasing number of observations, the probable degree of chance correlation is steadily reduced.

The number of variables correlating by chance increases as the number of observations decreases. In this study, for four parameter equations to be tested, the required number of observations to avoid undue risk of chance correlations can be insufficient. For these reasons, one parameter equations were sound models, and explained the data better.

The other one-term, two-term, three-term, four-term, and five-term equations were significant, but most of them have lower r values and higher s values than the equations given in *table III*. However, these one-term, two-term, three-term, four-term, and five-term equations contain at least one coefficient with a poor F statistic ($P > 0.05$).

4. Conclusion

The activity contributions of 3,4 disubstituted-1,2,4-oxa(thio)-diazole-5(4H)-one(thione) derivatives indicated that S containing compounds in position 1 have higher biological activities against some bacteria and fungal species. QSAR studies which concerned the quantum chemical, physical and structural parameters confirmed this result.

The highest correlation between the biological activities and structural parameter I_Y revealed that the main interactions between the binding sites should be on the lone pair electrons of oxygen and sulfur atoms in position 1 of the five membered ring of some 3,4 disubstituted-1,2,4 oxa(thio)-diazole-5(4 H)-one(thione) derivatives.

References

[1] Gümüs F., Dürüst Y., Dürüst N., Abbasoglu U., Pharmazie 48 (1993) 867–868.

[2] Greco G., Novellino E., Silipo C., Vittoria A., Quant. Struct.-Act. Relat. 11 (1992) 461–477.

[3] Mekenyan O.G., Veith G.D., Bradbury S.P., Russom C.L., Quan. Struct.-Act. Relat. 12 (1993) 132–136.

[4] Nakayama A., Hagiwara K., Hashimoto S., Shimoda S., Quant. Struct.-Act. Relat. 12 (1993) 251–255.

[5] Sener E., Turgut H., Yalçin Y., Ören Y., Türker L., Çelebi N., Akin A., Int. J. Pharm. 110 (1994) 109–115.

[6] Veith G.D., Mekenyan O.G., Quant. Struct.-Act. Relat. 12 (1993) 349–356.

[7] Agirbas H., Dürüst Y., Sümengen D., Sulfur and Silicon 66 (1992) 321–324.

[8] Dürüst Y., Agirbas H., Sümengen D., Phosphorus Sulfur and Silicon 62 (1991) 47–51.

[9] Sümengen D., Agirbas H., Dürüst Y., Dogan N., Chim. Acta Turc. 20 (1992) 17–23.

[10] Dewar M.J.S., Stewart J.J.P., Zocbisch E.G., Healy E.F., J. Am. Chem. Soc. 107 (1985) 3902–3909.

[11] Stewart J.J.P., MOPAC Manual, Frank J. Seiler Research Laboratory, United States Air Force Academy, USA, (1990).

[12] Hansch C., Leo A., Substituent Constants for Correlation Analysis in Chemistry and Biology, John Wiley and Sons, New York, 1979.

[13] Norusis M.J., The SPSS Guide to Data Analysis for SPSS/PC+ (2nd ed.), SPSS Inc., Chicago, 1991, pp. 849–872.

[14] Rawlings J.O., Applied Regression Analysis: A Research Tool, Wadsworth Inc., California, 1988, pp. 327–336.

[15] Anderson T.W., Stanley L.S., An Introduction to the Statistical Analysis of Data, Houghton Mifflin Company, Boston, 1978, pp. 619–637.

[16] Darper N.R., Smith H., Applied Regression Analysis, John Wiley and Sons Inc., New York, 1981, pp. 294–342.

[17] Howell D.C., Statistical Methods for Psychology, PWS-Kent Publishing Company, Boston, 1987, pp. 463–507.

[18] Norman H.I., Hull C.H., Jenkins J.G., Steinbrenner K., Bent D.H., SPSS Statistical Package for the Social Sciences, McGraw Hill Inc, New York, 1975, pp. 340–341.

[19] Wold S., Quant Struct.-Act. Relat. 10 (1991) 191–193.

[20] Topliss J.G., Costello, R.J., J. Med. Chem. 15 (1972) 1066–1068.