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Quantitative Structure–Activity Relationships (QSAR) of Two Series of *O*-Aryl or *N*-Aryl *O*-Ethyl Phosphoramidate and Phosphorodiamidate Fungicides Incorporating Amino Acid Ethyl Esters

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Structure-activity relationships of wide groups of fungicides have been examined e. g. aliphatic aldehydes, ketones and alcohols (Andersen et al. 1994), alicyclic diamines (Havis et al. 1996), substituted 2-phenylbenzofurans (Chamberlain and Carter 1980), hydroxamic acids (Bravo and Lazo 1996), heterocyclic nitrogen compounds (Chen et al. 1997) and some organophosphorus compounds (Cremlyn et al. 1974; Roy et al. 1996; Dureja et al. 1980). These quantitative structure activity relationships have been used to predict the toxicity of new compounds, correlate bioactivity of chemicals with their physicochemical properties and shed light on the nature of fungicide-bioreceptor interaction. Physicochemical descriptors used included the stereo-electronic parameters and the substituent parameters, π, characterizing hydrophobicity (Hansch and Leo 1979). It was also mentioned that attachment of an amino acid to a drug enhances its cellular uptake (Chen et al. 1994). Therefore, to extend the study of the biological effects and quantitative structure-activity relationships of pesticides and chemicals (Ali and Mostafa 1999; Ismail et al. 1993; Ali et al. 1999), a QSAR study has been performed to correlate the fungicidal activities of two series , *O*-aryl *O*-ethyl phosphoramidates and *N*-aryl *O*-ethyl phosphorodiamidates containing an amino acid ester moiety, with their electronic, steric and hydrophobic parameters.

MATERIALS AND METHODS

The phosphoramidates and diamidates used in this study (Fig. 1) were synthesized according to the method of (Ali and Mostafa 1999). Toxicological assessment of these compounds was performed by adding each compound to potato dextrose-agar medium in concentrations ranging from 0 (control) to 800 ppm. A minimum amount of ethanol was used when necessary for complete solubility; ethanol was added also to the control experiment in these cases. The fungi examined were *Fusarium solani, Rhizoctonia solani* and *Phytophthora parasitica*. All dishes were incubated at 25°C for seven days, then the diameter of each fungal colony was measured. Each experiment was repeated five times, then the mean values were used to compute the median effective molar concentrations, EC_{50}, by probit analysis.

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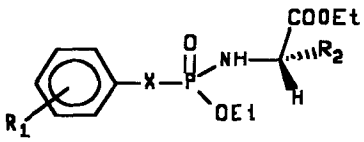

X	R_1	R_2	X	R_1	R_2
O	H	H (Gly) **1**	NH	H	H (Gly) **11**
O	H	Me (Ala) **2**	NH	H	Me (Ala) **12**
O	H	$PhCH_2$ (Phe) **3**	NH	H	$PhCH_2$ (Phe) **13**
O	H	$EtO_2C(CH_2)_2$ (Glu) **4**	NH	H	$EtO_2C(CH_2)_2$ (Glu) **14**
O	H	$MeS(CH_2)_2$ (Met) **5**	NH	H	$MeS(CH_2)_2$ (Met) **15**
O	*m*-Me	H (Gly) **6**	NH	*p*-Me	H (Gly) **16**
O	*m*-Cl	H (Gly) **7**	NH	*p*-OMe	H (Gly) **17**
O	*m*-NO_2	H (Gly) **8**	NH	*m*-Cl	H (Gly) **18**
O	*p*-NO_2	H (Gly) **9**	NH	*p*-Cl	H (Gly) **19**
O	*p*-NHCOMe	H (Gly) **10**	NH	*p*-CO_2Et	H (Gly) **20**

Figure 1. Structures of the phosphoramidates (**1-10**) and phosphorodiamidates (**11-20**) assessed in this study.

Stepwise multiple regression analysis was used to correlate the median effective molar concentrations of the tested compounds in each series with their polar (σ^*) and steric (Es) parameters of alkyl groups, R_2 (Taft 1956), the field parameter (F) of aryl substituents (R_1), the resonance parameter of *p*-aryl substituents (Swain et al. 1983), the molar refractivity of *m*- and *p*-aryl substituents (MR*m* and MR*p*, respectively), and the hydrophobicity parameter, π (Hansch and Leo 1979). Analysis was stopped after the introduction of two physicochemical parameters into the QSAR equations. The descriptor variables appeared in the equations were listed in Table 1. Equation adequacy was measured by the correlation coefficient (*R*), the standard error of estimates (*SE*), significance index for analysis of variance (*F*), and significance level (*p*). The contribution of each parameter to the toxicity variations was calculated from the square of the correlation coefficient (R^2) of each step.

RESULTS AND DISCUSSION

Quantitative structure-activity relationships (QSAR) are a valuable approach in determining the factors affecting toxicity of a series of chemicals, especially in the absence of known toxicity mechanism. According to the current acceptable target theory, the bioactivity of a chemical depends on two factors; the transport of the chemical from the outside aquatic medium to a particular target molecule in the organism's biophase and the reactivity of the chemical towards the target molecule (Hansch and Fujita 1964). The relationship between toxicity and these two factors is determined by the following equation.

Table 1. Descriptor variables of substituents used in the present correlations

Substituent	π	F	MR
H	0	0	1.03
Me	0.56	-0.01	5.65
Cl	0.71	0.72	6.03
NO_2	-0.28	1.00	7.36
NHCOMe	-0.97	0.77	14.93
OMe	-0.02	0.54	7.87
COOEt	0.51	0.47	17.47
$PhCH_2$	2.01	*	*

* not relevant to the present study

- *log toxicity* = *a log Kow* + *b log K* + *c*

Where *a* and *b* are coefficients, *c* is the equation constant, *Kow* is the partition coefficient of a chemical between 1-octanol and water, which represents the chemical penetration to reach the target molecule, and *K* is the equilibrium constant of the binding reaction between the toxicant and the target molecule, which reflects their stereo-electronic interaction (Zhao et al. 1993). However, the penetration process can also be represented by the hydrophobicity parameter, π, while the chemical's reactivity is best modeled by Taft's polar (σ*) and steric (Es) parameters of alkyl groups and the field (F) and resonance (R) parameters of aryl substituents. The steric bulk of aryl substituents was represented by the molar refractivity (MR). The advantage of using these physicochemical parameters is that they allow the study of the effect of each electronic and steric parameter separately on the toxicant-bioreceptor interaction. In addition, they are available in the literature without performing more experiments, which saves time and expenses. The negative logarithms of the EC_{50} of the two series of *N*-aryl *O*-ethyl phosphoramidates (**1-10**) and *O*-aryl *O*-ethyl phosphorodiamidates (**11-20**) on *Fusarium solani* and *Rhizoctonia solani* are listed in Table 2. Table 2 also shows that compounds **3, 9, 15, 18** and **19** were more effective fungicides toward *Fusarium solani* than the commercial fungicides, rizolex [*O*-(2,6-dichloro-4-methylphenyl)*O*,*O*-dimethyl phosphorothioate] and daconil [2,4,5,6-tetrachloro-1,3-dicyanobenzene] under the same experimental conditions. Although most compounds were effective towards *Fusarium solani* and *Rhizoctonia solani*, most of them were not effective (EC_{50} > 1000 ppm) towards *Phytophthora parasitica* indicating that these compounds possess some selectivity towards different microorganisms. Correlating toxicity of the phosphoramidates (**1-3, 6-10**) to *Fusarium solani* and *Rhizoctonia solani* with the hydrophobic and stereo-electronic parameters produced equations 1 and 2, respectively.

Table 2. The experimental and calculated toxicity of the phosphoramidates (**1-10**) and phosphorodiamidates (**11-20**)* to *Fusarium solani* and *Rhizoctonia solani*

Compound	*Fusarium* exptl.	eq.(1)	*Rhizoctonia* exptl.	eq.(2)	Compound	*Fusarium* exptl.	eq.(3)	*Rhizoctonia* exptl.	eq.(4)
1	3.428	3.703	2.798	2.766	**11**	3.646	3.692	2.671	2.812
2	3.509	4.258	2.764	2.766	**12**	3.713	3.692	2.833	2.812
3	4.077	5.693	2.766	2.766	**13**	3.671	3.692	2.658	2.812
4	4.042	-	2.734	-	**14**	3.747	-	2.823	-
5	4.058	-	2.745	-	**15**	4.117	-	2.811	-
6	4.028	4.637	2.534	2.589	**16**	3.777	3.690	3.289	2.954
7	4.206	4.816	2.742	2.642	**17**	3.790	3.822	3.125	3.351
8	4.252	3.945	2.579	2.617	**18**	4.236	4.236	3.345	3.238
9	3.981	3.426	2.837	2.858	**19**	3.956	3.866	3.482	3.398
10	3.677	2.743	2.820	2.837	**20**	3.706	3.805	3.589	3.616
Rizolex	3.721	-	3.972	-					
Daconil	3.853	-	3.311	-					

* - log EC_{50} (mol/L)

$$- \log EC_{50} = 3.619 + 0.082MRm + 0.099\pi \quad (1)$$
$n = 8$, $R = 0.771$, $SE = 0.238$, $F = 3.669$, $p = 0.272$

$$- \log EC_{50} = 2.805 - 0.038MRm + 0.092F \quad (2)$$
$n = 8$, $R = 0.973$, $SE = 0.069$, $F = 2.907$, $p = 0.002$

Equation 1 explained 59.48% of the toxicity variations; 51.65 and 7.83% of these variations were explained by MR*m* and π respectively.

Equation 2 explained 81.61% of the toxicity variations, 67.70 and 13.91% of which were due to MR*m* and F, respectively.

The physicochemical parameters of the phosphorodiamidates (**11-13, 16-20**) were correlated to the toxicity to *Fusarium* and *Rhizoctonia solani* as presented by equations 3 and 4, respectively.

$$- \log EC_{50} = 3.616 + 0.074MRm + 0.241F \quad (3)$$
$n = 8$, $R = 0.944$, $SE = 0.077$, $F = 20.331$, $p < 0.001$

$$- \log EC_{50} = 2.779 + 0.592F + 0.032MRp \quad (4)$$
$n = 8$, $R = 0.869$, $SE = 0.213$, $F = 7.719$, $p = 0.005$

Equation 3 explains 89.05% of the toxicity variations; 76.02 and 13.03% of these variations were explained by MR*m*, and F, respectively. Equation 4 explained 75.54% of the toxicity variations, 53.87 and 21.67% of which were due to F and MR*p*, respectively.

Equations 1-4 revealed some points; first, the toxicity of these compounds was mainly controlled by the steric and electronic effects of aryl substituents and the compounds hydrophobicity as represented by MR, F and π parameters respectively. Second, it appears from equations 1-4 that the steric bulk of aryl substituents as represented by the molar refractivity (MR) plays an important role during the interaction between the fungicidal molecule and the bioreceptor which is in consistence with the previous results (Roy et al. 1996) with other organophosphorus fungicides containing aryl groups. Third, the field parameter (F) in equations 2-4 showed that the toxicity was generally increased by the addition of strong electron accepting groups, which might suggest the nucleophilic attack of the bioreceptor on the partially positive phosphorus atom in the phosphoramidates and phosphorodiamidates during their interaction, similar to that observed between acetylcholinesterase and phosphoramidates (Ali and Mohamed 1999) or other organophosphorus inhibitors (Hassall 1990). Forth, all equations represented reasonable correlations between the toxicity and the physicochemical properties of toxicants, which reflects the reliability of this method to predict toxicity of untstudied compounds. The calculated toxicity (- log EC_{50}) by equations 1-4 were included in Table 2.

Table 2 also showed that incorporating different amino acid moiety other than glycine or alanine (R_2 = H and Me respectively) could improve toxicity in some instances. For example, the phosphoramidates **4, 5** and phosphorodiamidates **14, 15** containing Glu or Met moiety were more toxic to *Fusarium solani* than their analogous containing Gly or Ala moiety. Studying the point of incorporating different amino acid moieties are being undertaken.

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