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Potential Antifungal Activity and Structure–Activity Relationships of Some 2-Amino Acid Substituted Benzo-1,3,2-Dioxaphospholene, Oxazaphospholine and Diazaphospholine 2-Ones

H. M. Ali,[1] M. K. Ali[2]

[1] Department of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 11241
[2] Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 11241

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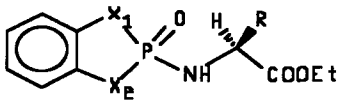
Application of various organophosphorus compounds as fungicides and their structure-activity relationships have been studied extensively e.g. *O,O*-bisaryl alkyl phosphonates (Roy et al. 1996), diaryl dichloromethyl phosphonodithioates (Roy and Mukerjee 1975), and alkyl phenyl (dichloromethyl) phosphonates (Dureja et al. 1980). Recently, groups of potential fungicides have been developed derived from diamines (Havis et al. 1996), *o*-phenylenediamines (Khan et al. 1995), and *o*-aminophenols (Bravo and Lazo 1996). It was also reported that incorporating an amino acid (Chen et al. 1994) or peptide moiety in drugs or toxic agents facilitates carrying these chemicals into the cells of the organisms, which leads, in the case of toxicants, to their intracellular accumulation causing cell death (Yadav et al. 1996; Menton et al. 1986). In addition, incorporating an amino acid moiety in toxicants is expected to yield nontoxic metabolites upon hydrolysis in plants or soils. Coupling the above information with our desire to develop effective agricultural pesticides (Ali and Mostafa 1999; Ismail et al. 1993; Ali and Mohamed 1999) prompted us to examine the recently reported 2-amino acid substituted benzo-1,3,2-dioxaphospholene, oxazaphospholine and diazaphospholine 2-ones (Ali 1999) as fungicides against *Alternaria cucumerina* and *Fusarium pallidoroseum* and study their structure-activity relationship.

MATERIALS AND METHODS

Benzo-1,3,2-dioxaphospholene, oxazaphospholine and diazaphospholine 2-ones used in this study (Figure 1) were synthesized according to the reported procedure (Ali 1999). Toxicological assessment of these compounds was performed by adding each compound to potato dextrose-agar medium in concentrations which ranged 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 *Alternaria cucumerina* and *Fusarium pallidoroseum*. All dishes were incubated at 25°C for seven days, then the diameter of each fungal colony assessed in this study 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. The atom superposition and electron

Correspondence to: H. M. Ali



X_1	X_2	R	X_1	X_2	R
O	O	H (Gly) **1**	O	N	$EtOOC-CH_2-CH_2$ (Glu) **9**
O	O	CH_3 (Ala) **2**	O	N	$CH_3-S-CH_2-CH_2$ (Met) **10**
O	O	$PhCH_2$ (Phe) **3**	N	N	H (Gly) **11**
O	O	$EtOOC-CH_2-CH_2$ (Glu) **4**	N	N	CH_3 (Ala) **12**
O	O	$CH_3-S-CH_2-CH_2$ (Met) **5**	N	N	CH_3 (Ala) **13**
O	N	H (Gly) **6**	N	N	$EtOOC-CH_2-CH_2$ (Glu) **14**
O	N	CH_3 (Ala) 7	N	N	$CH_3-S-CH_2-CH_2$ (Met) **15**
O	N	$PhCH_2$ (Phe) **8**			

Figure 1. Structures of the dioxaphospholene (**1-5**), oxazaphospholine (**6-10**) and diazaphospholine (**11-15**) series

delocalization molecular orbital (ASED-MO) method was applied by using standard parameters as a semiemperical method for molecular orbital calculations (Anderson et al. 1987).

RESULTS AND DISCUSSION

Three series of benzo-1,3,2-dioxaphospholene, oxazaphospholine and diazaphospholine 2-ones containing an amino acid substituted in the 2-position were examined as fungicides against *Alternaria cucumerina* and *Fusarium pallidoroseum*. The results are listed in Table 1. These compounds were synthesized recently in our laboratory and found to be strong acetyl-cholinesterase inhibitors (Ali 1999). Three commercial fungicides, tropsin [diethyl 4,4 -*O*-phenylenebis-(3-thioallophanate)], daconil [2,4,5,6-tetrachloro-1,3-benzenedinitrile], and vitavax-captan [5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide and *N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (3:2 w/w) respectively] were also tested under the same experimental condition and the toxicity values are included in Table 1. Toxicity to *Alternaria cucumerina* indicated that dioxaphospholenes **1**,**4** and **5** were more toxic (higher - log EC_{50} and - log EC_{90}) than the three commercial fungicides, while all other tested compounds were more toxic than daconil and vitavax-captan. Toxicity toward *Fusarium pallidoroseum* showed that the dioxaphospholene and oxazaphospholine series (**1-10**) were also more toxic than daconil and vitavax-captan as expressed by both EC_{50} and EC_{90}.

To study the structure-activity relationships of these compounds, the negative logarithm of the median effective concentration data of the three series to both

Table 1. Fungitoxicity data against *Alternaria cucumerina* and *Fusarium pallidoroseum**

Compound	*Alternaria cucumerina* - log EC_{50}	-log EC_{90}	*Fusarium pallidoroseum* - log EC_{50}	-log EC_{90}
1	5.13	4.31	4.58	3.60
2	4.73	3.97	4.58	3.82
3	4.72	3.33	4.62	3.80
4	5.03	4.06	4.58	3.67
5	5.03	4.05	4.50	3.45
6	4.76	3.98	4.29	3.10
7	4.56	3.73	4.28	3.47
8	4.49	2.95	4.33	3.29
9	4.52	3.02	4.33	3.86
10	4.57	3.28	4.25	3.21
11	4.28	3.10	4.11	3.09
12	4.30	3.25	4.10	3.58
13	4.25	3.22	3.98	3.14
14	4.40	3.58	4.01	3.11
15	4.31	3.28	3.88	3.15
Tropsin	4.78	3.91	4.96	4.02
Daconil	4.16	2.48	4.11	2.48
Vitavax-Captan	3.70	2.53	3.66	2.62

*- log EC_{50} or - log EC_{90} (mol/L)

fungi are presented graphically in Figure 2. It is clear from this figure that toxicity of the three series toward both fungi is in the order: dioxaphospholene > oxazaphospholine > diazaphospholine. This order of decreasing toxicity is consistent with the increasing number of nitrogen atoms around the phosphorus atom in the three series, which suggests an interaction of a nucleophilic center of the bioreceptor with the electrophilic phosphorus atom in the toxicant. Increasing the number of nitrogen atoms decreases the electrophilic character of the phosphorus atom by the overlapping between the phosphorus d_π orbital and the nitrogen p_π orbital and hence reduces toxicity (Garrison and Boozer 1968; Quistad et al. 1970) as shown in Figure 3. Molecular orbital calculations also showed that the P-N bond order in the dioxaphospholene **2** (0.475) is smaller than that in diazaphospholine **12** (0.519) which reflects the more double bond character of the P-N bond in the later series. Also, the P=O bond order in the dioxaphospholene **2** (0.6011) is smaller and thus has less double bond character than that in the diazaphospholine **12** (0.626) which facilitates the nucleophilic attack on the phosphorus atom and thus increases the toxicity of the dioxaphospholene series. The oxazaphospholine **12** gave close bond orders to that of the diazaphospholine **7**.

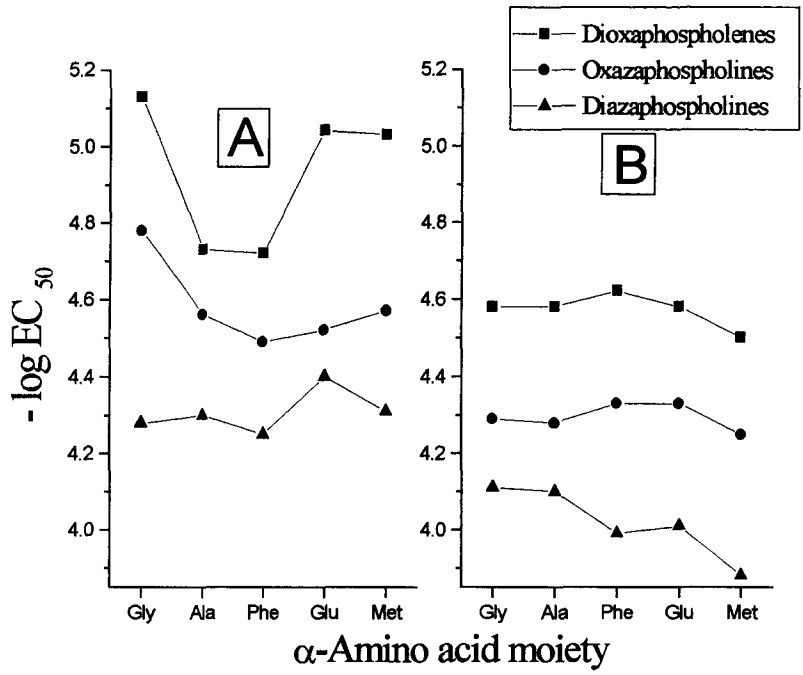


Figure 2. Toxicity of the dioxaphospholenes (**1-5**), oxazaphospholines (**6-10**) and diazaphospholines (**11-15**) against *Alternaria cucumerina* (A) and *Fusarium pallidoroseum* (B)

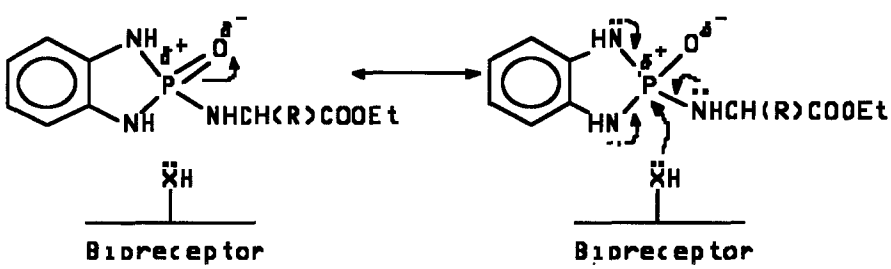


Figure 3. Reducing the fungitoxicity process by the nitrogen atoms around the phosphorus atom

This conclusion is also supported by our previous finding that the fungal toxicity of some *O*-aryl phosphoramidates and *N*-aryl phosphorodiamidates incorporating amino acid moiety can be increased by introducing strong electron withdrawing aryl substituents, which increases the phosphorus atom

electrophilicity (*Ali and Ali in press*). Figure 2 also indicates that the three series were more toxic toward *Alternaria cucumerina* than toward *Fusarium pallidoroseum*. Changing the amino acid moiety affected toxicity in all series especially in the dioxaphospholene series, which was explained previously (Roy et al. 1996) by a steric interaction between the bulk of alkyl groups in some organophosphours fungicides and the bioreceptor. This interaction can be maximized by changing the alkyl group in the amino acid moiety and thus improving the fungal toxicity of these compounds.

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