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# Synthesis, Pesticidal Activity And Quantitative Structure-Activity Relationships of Aseries of *N*-(2-oxido-1,3,2-benzodioxaphosphol-2-yl) Amino Acid Ethyl or Diethyl Esters

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**Abstract:** A few members of the title compounds showed previously good pesticidal activities in addition to their low mammalian toxicity. Therefore, a series of these compounds were prepared by the reaction of catechol, phosphoryl chloride and amino acid ethyl or diethyl esters (one-pot synthesis) to evaluate their pesticidal activities and examine the factors affecting their insecticidal and fungicidal toxicity. Toxicity against both mosquito ($LC_{50}$) and house fly ($LD_{50}$) larvae, and anticholinesterase activity (median inhibition concentration, $I_{50}$, and bimolecular rate constant, ki) were determined. Some of the examined compounds were stronger insect cholinesterase inhibitors than the commercial insecticide fenitrothion. Toxicity against both insect larvae showed strong positive correlations ($R \geq 0.951$) with either $I_{50}$ or ki, modeling the enzyme inhibition process, and the hydrophobicity parameter (logP), modeling the penetration process. The prepared compounds were more toxic against *Rizoctonia solani* than *Fuzarium solani* where some compounds showed higher toxicity towards *Rizoctonia solani* than that of the commercial fungicides rizolex and daconil.

**Keywords:** 1,3,2-benzodioxaphospholenes; insecticidal activity; fungicidal activity; anticholinesterase; QSAR.

## INTRODUCTION

Much effort has widely been paid to the synthesis of new groups of organophosphorus pesticides in order to define compounds with acceptable pesticidal activity and low adverse effects on environments. Although the synthesis of some 1,3,2-dioxaphospholene derivatives has been described previously (Ramirez and Marecek 1984 ; Chasar 1987 ; Goerg *et al*. 1997 ; Mironov *et al*. 1999), their biological activities, especially as pesticides, still remain not explored. However, the insecticidal activities of some related ring systems e.g. 1,3,2-oxazaphospholidine and benzodioxaphosphorin 2-sulfides (Wu *et al*. 1987a,b), 4-H-1,3,2-benzoxazaphosphorines (Yoshikawa *et al*. 1986), 4H-1,3,2-benzodioxaphosphorins (Eto *et al*. 1981), 2-methoxy-5-phenyl-1,3,2-oxazaphospholidine 2-sulfide (Wu *et al*. 1989), and 2, 4 (and/or 5) substituted oxazaphospholidines, oxathiaphospholanes, and thiazaphospholidines (Wu *et al*. 1988) have been reported. Recently, in our laboratory, few members of *N*-(2-oxido-1,3,2-benzodioxaphosphol-2-yl) amino acid ethyl esters have been introduced (Ali 1999). These compounds showed *in vivo* lower effects on acetylcholinesterase and lower neurotoxicity to mammals than those caused by fenitrothion, a commercial insecticide with reported moderate toxicity to mammals (Ali *et al*. 2004); in addition, they showed promising pesticidal activity (Ali 1999 ; Ali and Ali 2000). Their low mammalian toxicity was attributed to their low persistence and giving non-toxic metabolites (Ali *et al*. 2003). To examine the factors affecting their insecticidal and fungicidal activities, new members of the title series were synthesized and their activities were correlated quantitatively with their structures.

## MATERIALS AND METHODS

### *Chemicals and Instrumentation:*

Chemicals were reagent grade. Solvents were freshly distilled before use. Catechol was purified by crystallization from benzene. Phosphoryl chloride was freshly purified by fractional distillation. Except for

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glycine, α–amino acids were of L-(S)-configuration. Fenitrothion, *O*,*O*-dimethyl-*O*-(3-methyl-4- nitrophenyl) phosphorothioate, and rizolex, *O*-(2,6-dichloro-4-methylphenyl) *O*,*O*-dimethyl phosphorothioate, were obtained from Sumitomo Chemical Co., while daconil, 2,4,5,6-tetrachloro-1,3-dicyanobenzene, was obtained from Syngenta Co. Title compounds were dissolved in $CDCl_3$/TFAA and analyzed by NMR Burker-300 spectrophotometer using TMS as internal standard. IR spectra were recorded on a Nicolet 460 FT-IR spectrophotometer. Electronic absorption was recorded on Shimatzu 160 A spectrophotometer. Purity was checked by 12A Shimatzu GC equipped with electron capture detector. The injection and detector temperatures were 230 and 250°C, respectively. The column was packed with chromosorb Q coated with 2 % dexil. Column temperature was isothermal at 180°C.

***Synthesis of Benzo-1,3,2-dioxaphospholene-2-one Derivatives 1-11:***

A series of *N*-(2-oxido-1,3,2-benzodioxaphosphol-2-yl) glycine, alanine, valine, norleucine, leucine, isoleucine, aspartic acid, asparagine, glutamic acid, methionine and phenylalanine ethyl or diethyl esters, 1-11 respectively, were prepared by the method previously published (Ali 1999).A solution of 5.5 g (0.05 mol) catechol and 10.1 g (0.1 mol) triethylamine in THF was added slowly to an ice –cooled solution of 7.7 g (0.05 mol) phosphoryl chloride dissolved in 60 mL THF-benzene (2:1 v/v). After stirring for 3 hours, a mixture of 0.05 mol of each of triethylamine and a suitable α -amino acid ethyl ester was added. The mixture was stirred for 2 more hours then worked up. The crude product was purified on a silica gel column using $CH_2Cl_2$/MeOH as a solvent system. Yields were 60-70% with purity > 97%.

***Toxicological Measurements:***

Toxicity towards mosquito larvae (*Cules pupiens* L.) was determined by applying the WHO procedure (Anonymous 1981).Each compound dissolved in ethanol (1.0 ml) was added to 250 mL water to prepare a final concentration in the range of 0.02 - 0.5 mM. One mL ethanol was added in the control test. About 20-25 mosquito larvae were used in each experiment. Mortality was counted after 48 hours. Each experiment was performed in triplicate and the means were used to calculate the lethal concentrations in mM.

Toxicity towards house fly larvae (*Musca domestica* L.) was determined by adopting residual film method (Busvine 1971).Each compound dissolved in acetone (1.0 mL) was placed in a Petri dish (11 cm in diameter). Concentrations prepared were 0.02-0.50 mM; one mL acetone was used in a control experiment. Solutions were spread on the entire dish surface then the solvent was evaporated at room temperature. Ten 3rd-instar house fly larvae were added to each Petri dish and kept at 25-30°C and 60-70% RH. Mortality rate was estimated after 24 hours. Each experiment was performed in triplicate to calculate the lethal doses in m mole / $cm^2$.

Toxicity index was calculated by the formula: toxicity of the most effective compound X 100 / toxicity of a compound.

Acetylcholinesterase, AChE (EC 3.1.1.7), was extracted by removal of house fly heads from insects then immersed immediately in ice-cooled 0.1 M phosphate buffer (pH 7.0) containing 0.5% triton-X-100 to assist solubilizing membrane-bound AChE and 0.25 M sucrose to maintain the osmotic potential during the assays. Heads were homogenized in cold buffer in the ratio 10 heads/ml buffer in a glass homogenizer. The homogenate was centrifuged at 4°C for 15 minutes and the supernatant was used as insect AChE. The enzyme activity was determined as mentioned with Ellman *et al.* (1961).

Fungicidal activity was determined by adding each compound, dissolved in ethanol (1.0 mL), to potato dextrose-agar medium in final concentrations ranged from 0 (control) to 2.7 mM. Fungi examined were *Fusarium solani* and *Rhizoctonia solani*. All dishes were incubated at 25°C for 24 hours, then colonies' diameters were measured. Each experiment was performed in triplicate and the mean values were used to compute the effective concentrations in mM.

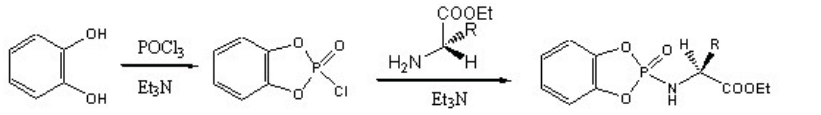
***Statistics and Calculations:***

Statistical analyses were performed by the statistical package SPSS version 11.5.The lethal concentrations ($LC_{50}$ and $LC_{90}$), lethal doses ($LD_{50}$ and $LD_{90}$) and effective concentrations ($EC_{50}$ and $EC_{90}$) were computed by probit analysis. The median inhibition concentration ($I_{50}$), in µM, was calculated by linear regression analysis of AChE activity *versus* the compound concentrations. Energy minimization, Wang-Ford partial charges ($\rho^+$) and molar refractivity (MR) were computed by using CS Chem3D version 5.0 for MOPAC calculations. The hydrophobicity parameter logP was also involved in regressions (Viswanadhan *et al.* 1989). The inhibition rate constant (ki) of the prepared compounds was determined previously (Ali et al. 2005). Equations were justified by the correlation coefficient (R), the standard error of estimates (SE) and significance level (p).

## RESULTS AND DISCUSSIONS

***Synthesis and Structures:***

A series of *N*-(2-oxido-1,3,2-benzodioxaphosphol-2-yl) amino acid ethyl or diethyl esters (1-11) was prepared by phsphorylation of catechol with phosphoryl chloride in the presence of triethylamine as a base to yield, *in situ*, benzo-1,3,2-dioxaphospholene-2-chloro-2-ones. Dilution with benzene or THF minimized the polymerization side reaction. The product reacted with various amino acid ethyl or diethyl esters to give the titled compounds in a one-pot synthesis as presented in Figure 1.




R = H (Gly) **1**
= Me (Ala) **2**
= i-Pr (Val) **3**
= n-Bu (Nle) **4**
= i-Bu (Leu) **5**
= s-Bu (Ile) **6**

R = $CH_2COOEt$ (Asp) **7**
= $CH_2CONH_2$ (Asn) **8**
= $CH_2CH_2COOEt$ (Glu) **9**
= $CH_2CH_2SMe$ (Met) **10**
= $CH_2Ph$ (Phe) **11**

**Fig. 1:** Synthesis and structures of the *N*-(2-oxido-1,3,2-benzodioxaphosphol-2- yl) amino acid esters.

**Table 1:** Spectroscopic data of the prepared benzodioxaphospholenes.

| | $^1$H NMR data | | | |
|---|---|---|---|---|
| Compound | (4H) aromatic | NH | $OCH_2CH_3$ | relative to NH group |
| 3 | 6.90(s) | 6.56(m) | 4.09(2H,q),1.21(3H,t) | 3.71(α-CH,m),1.76(β-CH,m), 0.99 (γ-2Me,d) |
| 4 | 6.89(s) | 6.70(m) | 4.02(2H,q),1.26(3H,t) | 3.71(α-CH,m),1.77(β-$CH_2$,m), 1.44(γ-$CH_2$,m), 1.26(δ-2$CH_2$,m),0.84(6-$CH_3$,t) |
| 5 | 6.85(s) | 6.24(m) | 4.21(2H,q),1.29(3H,t) | 4.00(α-CH,m),1.80(β-$CH_2$,m), 1.64(γ-CH,m), 0.97(δ-2Me,d,J=5.88) |
| 6 | 6.89(s) | 6.80(m) | 4.17(2H,q),1.28(3H,t) | 3.98(α-CH,m), 1.83(β-CH,m), 1.32(γ-$CH_2$,m), 1.02(γ-Me,d),0.93(δ-Me,t) |
| 7 | 6.88(s) | 6.44(m) | 4.07(4H,q),1.91(6H,m) | 3.81 (α-CH,m), 3.53(β-$CH_2$,d,J=7.8) |
| 8 | 6.89(s) | 6.70(m) | 4.09(2H,q),1.28(3H,t) | 3.72(α-CH,m), 3.53(β-$CH_2$,d,J=6.39), 5.70($NH_2$,broad) |

| | IR data | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Aromatic | | | | |
| Compound | N-H str | C=O str | C=C str | C-H out-of-plane | P=O str | P-N str | P-O str |
| 3 | 3219 | 1737 | 1603, 1469 | 752 | 1239 | 1032 | 923 |
| 4 | 3194 | 1740 | 1591, 1498 | 746 | 1219 | 1055 | 922 |
| 5 | 3188 | 1740 | 1587, 1516 | 751 | 1220 | 1043 | 922 |
| 6 | 3217 | 1737 | 1598, 1468 | 747 | 1220 | 1034 | 921 |
| 7 | 3203 | 17361 | 603, 1470 | 750 | 1220 | 1037 | 922 |
| 8 | 3228 | 1734 | 1610, 1471 | 752 | 1207 | 1014 | 920 |

The $^1$H NMR data of the new dioxaphospholenes (3-8) are listed in Table 1. Spectra showed that the four aromatic protons gave a singlet at 6.85-6.90 ppm because of the similarity of both ring substituents. The amino protons were deshielded at 624-6.80 ppm by the effect of the P=O group, and appeared as multiplets because of the splitting by neighboring phosphorus and hydrogen atoms. The methylene protons of Nle (4), Ile (6), Asp (7) and Asn (8) moieties appeared as multiplets since they are diasteriotopic and thus they split each other signals and split further by the nearby protons.

IR spectra presented in Table 1 showed that the absorptions of both the carbonyl and amino groups had low frequencies (1740-1734 and 3228-3188 $cm^{-1}$ respectively) and low intensities. This result suggested hydrogen bonding between the carbonyl oxygen and amino hydrogen atoms in a five-member ring (Ali 1999) which was also confirmed by MOPAC calculations for energy minimization. The resulting models showed that the afore-mentioned oxygen and hydrogen atoms are in a suitable orientation and distance (~ 2.69 Å) for hydrogen bonding.

***Insecticidal Activity:***

Toxicity data of compounds 1-11 are presented in Table 2. Insecticidal activity against mosquito larvae was determined as the lethal concentrations ($LC_{50}$ and $LC_{90}$), in mM, while toxicity against house fly larvae was determined as the lethal doses ($LD_{50}$ and $LD_{90}$) and expressed in m mole deposited per $cm^2$ of glass surface, since the deposit rate showed linear relationship with the true dose entering the insect (Busvine 1971). Anticholinesterase activity expressed as $I_{50}$ indicated that compounds 4, 5, 6, 10 and 11 were stronger AChE inhibitors than the commercial pesticide, fenitrothion, which showed higher $I_{50}$, 393.855 µM, under our experimental conditions.

**Table 2:** Insecticidal activity of the prepared benzodioxaphospholenes.

| Compound | $I_{50}$[a] | ki[b] | Mosquito Larvae[c] | | | | House fly larvae[d] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $LC_{50}$ | $LC_{50}$ (calc) | $LC_{90}$ | Toxicity Index | $LD_{50}$ | $LD_{50}$ (calc) | $LD_{90}$ | Toxicity Index |
| 1 | 626.92 | 2157.40 | 0.207 | 0.209 | 0.423 | 0.081 | 15.895 | 13.97 | 6.663 | 0.089 |
| 2 | 614.58 | 2560.82 | 0.159 | 0.233 | 0.611 | 0.073 | 14.221 | 15.31 | 6.011 | 0.082 |
| 3 | 453.70 | 2921.00 | 0.152 | 0.137 | 0.306 | 0.124 | 7.895 | 9.15 | 2.789 | 0.137 |
| 4 | 320.51 | 4033.28 | 0.075 | 0.062 | 0.212 | 0.274 | 3.579 | 4.31 | 2.727 | 0.290 |
| 5 | 195.91 | 7038.00 | 0.011 | 0.017 | 0.278 | 1.000 | | 1.25 | | 1.000 |
| 6 | 344.92 | 3467.94 | 0.088 | 0.075 | 0.320 | 0.227 | 6.361 | 5.16 | 2.253 | 0.242 |
| 7 | 650.86 | 1912.68 | 0.202 | 0.252 | 0.438 | 0.067 | | 16.55 | | 0.076 |
| 8 | 420.25 | 2966.54 | 0.061 | 0.054 | 0.184 | 0.315 | 4.001 | 4.05 | 2.189 | 0.309 |
| 9 | 453.76 | 3151.92 | 0.136 | 0.105 | 0.461 | 0.162 | 7.368 | 7.27 | 3.168 | 0.172 |
| 10 | 386.11 | 3409.52 | 0.105 | 0.081 | 0.401 | 0.210 | | 5.60 | | 0.223 |
| 11 | 332.40 | 3545.68 | 0.075 | 0.078 | 0.288 | 0.218 | 5.789 | 5.26 | 2.884 | 0.238 |

[a] $I_{50}$ in µM ($R > 0.95$)
[b] ki in $M^{-1}min^{-1}$
[c] $LC_{50}$ and $LC_{90}$ in mM, $LC_{50}$ (calc) estimated from eq. 1, toxicity index estimated from $LC_{50}$ calc.
[d] $LD_{50}$ ($X10^7$) and $LD_{90}$ ($X10^6$) in m mole / $cm^2$, $LD_{50}$ (calc) estimated from eq. 2, toxicity index estimated from $LD_{50}$ calc.

According to the target theory, the bioactivity of a compound depends on the ease of its transport through the organism's biophase and the reactivity of such compound towards the target molecule (Hansch and Fujita 1964).Therefore, to explain the variations in the toxicity against mosquito and house fly larvae, the toxicity was correlated by linear regression analysis to $I_{50}$, to model the enzyme-inhibitor interaction, and logP to model the compound penetration process to reach AChE active site; the results are presented in equations 1 and 2 respectively.

- $\text{Log } 1/LC_{50} = 8.194\ (0.876) + 2.632\ (0.308) \log 1/I_{50} - 0.133\ (0.050)\ \text{logP}$ (1)
  $R = 0.956,\ n = 11,\ SE = 0.117,\ p < 0.01$
- $\text{Log } 1/LD_{50} = 12.878\ (0.823) + 2.464\ (0.296) \log 1/I_{50} - 0.115\ (0.031)\ \text{logP}$ (2)
  $R = 0.967,\ n = 8,\ SE = 0.070,\ p < 0.01$

Equations 1 and 2 explained 91.4 and 93.5% of the toxicity variations respectively, 83.5 and 76.2% of which were due to $I_{50}$ while 7.9 and 17.3% were due to logP respectively. Compounds' reactivity towards AChE active site could also be represented by the bimolecular inhibition rate constant, ki (shown in Table 2) as presented by equations 3 and 4 for mosquito and house fly toxicity respectively.

- $\text{Log } 1/LC_{50} = 0.250\ (0.091) + 2.77 \times 10^{-4}\ (2.74 \times 10^{-5})\ ki - 0.075\ (0.039)\ \text{logP}$ (3)
  $R = 0.968,\ n = 11,\ SE = 0.101,\ p < 0.01$
- $\text{Log } 1/LD_{50} = 4.906\ (0.184) + 4.86 \times 10^{-4}\ (7.24 \times 10^{-5})\ ki - 0.129\ (0.040)\ \text{logP}$ (4)
  $R = 0.951,\ n = 8,\ SE = 0.085,\ p < 0.01$

The bimolecular inhibition rate constant contributes 90.8 and 70.2%, while the hydrophobicity parameter contributes 2.9 and 20.2% of the toxicity variations against mosquito (equation 3) and house fly (equation 4) larvae respectively. The results are consistent with those obtained by equations 1 and 2. The strong correlations not only explain the variations and trends of compounds' toxicity but also reflect the validity of using either $I_{50}$ or ki and the physicochemical parameters to model the toxicity of new compounds.

***Fungicidal Activity:***

The fungitoxicity of the prepared dioxaphospholenes presented in Table 3 showed that the compounds were more toxic against *Rizoctonia solani* ($EC_{50}$ 0.295-1.080 mM) than *Fuzarium solani* ($EC_{50}$ 1.303-2.373 mM); moreover, many compounds showed higher toxicity towards *Rizoctonia solani* than did the commercial fungicides rizolex and daconil ($EC_{50}$ 1.067 and 0.776 mM respectively). Previous results indicated that members of the prepared dioxaphospholenes (1, 2, 9, 10, 11) showed more fungicidal activity than that of their oxazaphospholine analogues which were in turn more toxic than their diazaphospholine analogues (Ali and Ali 2000). This order matches the increasing in the number of nitrogen atoms around the phosphorus atom, which decreases the phosphorus atom electrophilicity because of the overlapping between the nitrogen $p_\pi$ orbital and the phosphorus $d_\pi$ orbital and hence reduces reactivity (Quistad 1970; Ali and Ali 2000). Therefore, correlating the toxicity towards *Rizoctonia solani* ($EC_{50}$) with the partial charge of the phosphorus atoms ($\rho^+$) of the prepared compounds (equation 5) showed that the fungitoxicity is mainly controlled by the phosphorus atom electrophilicity and indicated a possible nucleophilic attack of the fungus bioreceptor towards the electrophilic phosphorus atom.

**Table 3:** Fungicidal activity of the prepared benzodioxaphospholenes.

| Compound | *Rizoctonia solani*[a] | | *Fuzarium solani*[a] | |
|---|---|---|---|---|
| | $EC_{50}$ | $EC_{90}$ | $EC_{50}$ | $EC_{90}$ |
| 1 | 0.661 | 2.423 | 2.204 | 3.445 |
| 2 | 1.080 | 2.209 | 2.373 | 3.118 |
| 3 | 0.295 | 1.793 | 1.766 | 2.403 |
| 4 | 0.902 | 3.077 | 1.513 | 2.724 |
| 5 | 1.014 | 2.638 | 1.866 | 2.410 |
| 6 | 0.793 | 1.50 | 91.508 | 2.413 |
| 7 | 0.627 | 1.486 | 1.683 | 2.575 |
| 8 | 0.911 | 2.057 | 1.926 | 2.685 |
| 9 | 0.386 | 1.380 | 1.460 | 2.077 |
| 10 | 0.522 | 1.457 | 1.303 | 1.908 |
| 11 | 0.804 | 2.479 | 1.738 | 2.704 |
| Rizolex | 1.067 | | 0.190 | |
| Daconil | 0.776 | | 0.140 | |

[a] $EC_{50}$ and $EC_{90}$ in mM

- $EC_{50} = 2.034\ (0.345) - 0.535\ (0.139)\ \rho^+(5)$
  $R = 0.789,\ n = 11,\ SE = 0.164,\ p < 0.01$

However, toxicity towards *Fuzarium solani* showed low correlation with the phosphorus electrophilicity (R = 0.354) but rather correlated with the molar refractivity, MR, of amino acid alkyl groups.

- $EC_{50} = 2.280\ (0.139) - 0.278\ (0.073)\ MR(6)$
  $R = 0.802,\ n = 10,\ SE = 0.190,\ p < 0.01$

These results explained the low toxicity of the prepared compounds towards *Fuzarium solani* which is less affected by compound reactivity and is more sensitive to the steric and polarization effects modeled by MR. Correlation between the fungicidal activity of some other phosphoramidates and MR was also previously observed (Ali and Ali 2000).

***Conclusions:***

The prepared dioxaphospholenes were derived from relatively non-toxic materials e.g. catechol, and amino acid ethyl or diethyl esters. Some compounds showed better anticholinesterase and fungicidal activities than

some commercial pesticides. Their insecticidal activity against both the examined insects depended mainly on their reactivity towards AChE and at lesser extend on their hydrophobicity. On the other hand, factors affecting their fungitoxicity differed depending on the examined fungus. Fungitoxicity against the sensitive fungus, *Rizoctonia solani*, depended on compounds' electrophilicity suggesting a nucleophilic attack of the fungus bioreceptor, while fungitoxicity against the resistant fungus, *Fuzarium solani*, depended mainly on the steric effect of the amino acid alkyl groups suggesting the involvement of the steric factor in the resistance process.

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