This article was downloaded by: [University of Otago] On: 17 July 2015, At: 08:39 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: 5 Howick Place, London, SW1P 1WG



Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information: <u>http://www.tandfonline.com/loi/gpss20</u>

SYNTHESIS AND QUANTITATIVE OF STRUCTURE-ACTIVITY RELATIONSHIPS OF PHOSPHORAMIDATES AND PHOSPHORODIAMIDATES INCORPORATING AMINO ACID ESTERS

Hussein M. Ali^a & Zidan H. Zidan^b

^a Agricultural Biochemistry Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 11241

^b Plant Protection Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 11241

Published online: 04 Oct 2006.

To cite this article: Hussein M. Ali & Zidan H. Zidan (2000) SYNTHESIS AND QUANTITATIVE OF STRUCTURE-ACTIVITY RELATIONSHIPS OF PHOSPHORAMIDATES AND PHOSPHORODIAMIDATES INCORPORATING AMINO ACID ESTERS, Phosphorus, Sulfur, and Silicon and the Related Elements, 163:1, 41-54, DOI: <u>10.1080/10426500008046609</u>

To link to this article: <u>http://dx.doi.org/10.1080/10426500008046609</u>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness,

or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <u>http://www.tandfonline.com/page/terms-and-conditions</u> Phosphorus, Sulfur and Silicon, 2000, Vol. 163, pp. 41-54 Reprints available directly from the publisher Photocopying permitted by license only © 2000 OPA (Overseas Publishers Association) Amsterdam N. V. Published under license by the Gordon and Breach Science Publishers imprint. Printed in Malaysia

SYNTHESIS AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF PHOSPHORAMIDATES AND PHOSPHORODIAMIDATES INCORPORATING AMINO ACID ESTERS

HUSSEIN M. ALI^{a*} and ZIDAN H. ZIDAN^b

^aAgricultural Biochemistry Department and ^bPlant Protection Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 11241

(Received May 27, 1999; In final form October 21, 1999)

Two series of O-aryl and N-aryl O-ethyl phosphoramidates and phosphorodiamidates respectively containing α -amino acid ester moleties have been synthesized and characterized by ¹H NMR, IR and mass spectroscopy. Stepwise multiple regression analysis showed that the anticholinesterase activity was strongly correlated with the chemical structures represented by the stereo-electronic and hydrophobic parameters with correlation coefficient of 0.999. These results revealed that the inhibition activity of both series was inversely correlated with the steric bulk of the p-aryl substituents and directly with the bulk of the alkyl groups of the amino acid moieties, whereas *m*-aryl substituents have no steric effect on the inhibition process. The inhibition was enhanced by strong π -electron acceptor aryl substituents and reduced by electron donating alkyl groups of the amino acids. This supported the proposed inhibition mechanism of nucleophilic attack of a hydroxyl group at the enzyme active site on the partially positive phosphorus atom in organophosphorus compounds. The inhibition was also increased by more hydrophilic substituents. These results showed the importance of both the reactivity of these compounds and their steric interaction with the AChE active site in controlling enzyme inhibition, in addition to the ease of more hydrophobic compounds to reach the enzyme active site.

Keywords: Synthesis; anticholinesterase; phosphoramidates; quantitative structure-activity relationship

^{*} Author to whom correspondence should be addressed.

INTRODUCTION

Phosphoramidates and phosphorodiamidates exhibited diverse biological activities. Their synthesis starts from phosphoryl chloride or its derivatives¹. Their acid hydrolysis² and P-N bond cleavage³ were investigated earlier. Incorporating amino acid ester derivatives⁴ or a 1, 3, 5, 2-triazaphosphorine ring⁵ in their structure showed antitumor activity. O-Alkyl O-(1-chlorophenyl-2-bromo or chloro) vinyl N, N-dialkyl-amidophosphates were tested in rats for their structure-oral toxicity relations.⁶ O-Ethyl O-5-methoxy-2-nitrophenyl⁷ N-alkyl, O-4-methyl-7-hydroxycoumarin N, N-dialkyl and O, O-dialkyl⁸ N- $(\beta$ -phthalimidoalkyl)⁹ or (sulfanilamide)¹⁰ phosphoramidothioates exhibited some fungicidal activity. O-2-isopropoxycarbonylphenyl **O-Ethyl** N-alkylphosphoramidates showed anticholinesterase activity and toxicity to adzuki bean weevil and bovine serum.¹¹ Synthesis and insecticidal activity of some cyclic phosphoramidates were also described.¹²

We have shown previously that phosphoramidates and diamidates containing amino acid ester moieties exhibited anticholinesterase activity.¹³ It was also found that electronic factor affects both the phosphoramidates toxicity and anticholinesterase activity.¹⁴ Therefore, in this article, two series of *O*-aryl and N-aryl *O*-ethyl phosphoramidates and phosphorodiamidates containing α -amino acid ester moieties were synthesized to study the effect of both electronic and steric factors of phenyl substituents and alkyl groups of different amino acid ester moieties on serum acetylcholinesterase activity and toxicity to mosquito larvae.

RESULTS AND DISCUSSION

The phosphoramidates and diamidates were synthesized by stepwise addition of substituted phenol or aniline, respectively and then an amino acid ester to *O*-ethyl phosphorodichloridate in the presence of triethylamine as a base according to the following scheme.

The characteristic IR bands of these compounds include $v_{P=O}at \sim 1230$, v_{P-OEt} at ~ 1040, v_{P-N} at ~ 970, $v_{PX-C(Ar)}$ at ~ 1280 and $v_{C=O}$ which varied from 1730–1750 cm⁻¹. This variation could be attributed to the participation in hydrogen bonding with the neighboring NH group *via* a five membered ring. The main IR vibration bands and elemental analyses data of all compounds are listed in Table I. The methylene protons of the glycine moiety appear in the ¹H NMR spectra as a doublets of doublets or multi-



plets because of the splitting with the neighboring NH proton and the phosphorus atom. The methine proton in the other amino acid moieties appears as multiplets because of the additional coupling with the adjacent methylene or methyl protons. The methylene protons in phenylalanine, glutamic and methionine moieties are diastereotopic; therefore, they couple each other and neighboring protons to appear as multiplets. The detailed features of ¹H NMR spectra of these compounds are summarized in Table II. Mass spectra of compounds 7 and 11, as examples, showed that the molecular ion peak (M⁺ 321 or 286 respectively) is absent or weak. The base peak results from hydrogen atom migration from the ethyl group to the ortho position of the phenyl ring (eight member ring transition state) to give ions at 128 in case of the phosphoramidate 7 or 93 in case of the diamidate 11. These ions lose CO or HCN molecules to give ions at m/e 100 or 66 respectively. A similar rearrangement was previously observed with the O-ethyl phosphorothioate, profenofos.²⁰ Losing an HCl molecule from compound 7 gives an ion at m/e 285 which undergoes the previous rearrangement to give an ion at m/e 66. Compound 11 also gives peak at 209 for M-Ph. These fragmentations and rearrangements are presented in Scheme I.

Inhibition of serum acetylcholinesterase (AChE) by the phosphoramidates (1-10) and phosphorodiamidates (11-20) are listed in Table III. It



was found previously that a number of O-ethyl O-2-isopropoxycarbonylphenyl N-alkykphosphoramidates and amidothioates were generally poor inhibitors of serum AChE, where the inhibition percentage ranged from 0 to 45 % at inhibitor concentration > 30 ppm.²¹However, AChE inhibition by the synthesized compounds at concentration level of 1 ppm rose to 73 % as shown in Table III. To study the stereo-electronic and the hydrophobic effects of the aryl (R_1) and the alkyl (R_2) substituents on the acetylcholinesterase inhibition process, in both the phosphoramidate and phosphorodiamidate series, the enzyme inhibition was correlated with the physicochemical parameters of R_1 and R_2 by using multiple regression analysis. The electronic factors of aryl substituents, R1, were modeled by both the resonance parameter (\mathbf{R}) of *p*-substituents and the field parameter (F) of m- and p-substituents. The steric factor was represented by the molar refractivity of m- and p substituents designated MRm and MRp, respectively. The stereo-electronic effects of alkyl groups (R_1) were best modeled by Taft's steric (Es) and polar (σ^*) parameters. The hydrophobic parameter, π , is usually used to reflect the penetration of toxicants to reach the enzyme active site. Correlating AChE inhibition with the physicochemical parameters of aryl substituents (R_1) in phosphoramidates (1, 6-10) and phosphorodiamidates (11, 16-20) gave a strong correlation as represented by equations 1 and 2, respectively.

Log%AChE inhibition = (1)

$$1.612 + 0.194R - 0.226\pi - 0.015 MRp - 0.104F$$

 $(\pm 0.013) (\pm 0.011) (\pm 0.019) (\pm 0.002) (\pm 0.017)$
 $n = 6, R = 0.999, s = 0.015, F = 132.830$

The parameters appeared in the equation contributed 71.99, 7.75, 13.34 and 6.73 % respectively of the AChE inhibition variations

Log%AChE inhibition = (2)
1.884 - 0.023 MRp + 0.189R - 0.199
$$\pi$$
 - 0.072F
(±0.019) (±0.001) (±0.014) (±0.034) (±0.030)
 $n = 6, R = 0.999, s = 0.018, F = 113.521$

The parameters appeared in the equation contributed 40.65, 41.47, 16.41 and 1.25 % respectively of the AChE inhibition variations

To study the effect of α -alkyl substituents of α -amino acid moieties (R₂) on enzyme inhibition, compounds **2**, **3** and compounds **12**, **13** were added to the regression analysis of the phosphoramidate and phosphorodiamidate series, which yielded an excellent correlation as represented by equations 3 and 4, respectively.

Log%AChE inhibition = (3) 1.298 + 0.194R + 1.994 σ^* - 0.535Es - 0.226 π - 0.015 MRp - 0.104F (±0.019)(±0.011)(±0.099) (±0.037) (±0.019) (±0.002) (±0.017) n = 8, R = 0.999, s = 0.015, F = 157.275

The parameters appeared in the equation contributed 37.54, 38.05, 8.63, 4.37, 7.51 and 3.79 % respectively of the AChE inhibition variations

Log%AChE inhibition = (4) 1.529 - 0.060Es - 0.023 MRp + 0.189R - 0.072F - 0.199 π + 0.745 σ^* (±0.027)(±0.062) (±0.001) (±0.014) (±0.030) (±0.034) (±0.152) n = 8, R = 0.999, s = 0.018, F = 99.633

The parameters appeared in the equation contributed 23.11, 31.51, 31.72, 7.73, 1.76 and 4.00 % respectively of the AChE inhibition variations.

15
20
July
17
t 08:39
] a
Otago
of
ersity
[Univ
by
ded
nloa
[MO
Ц

TABLE I Main IR vibration bands and elemental analyses data of phosphoramidates and phosphorodiamidates

46

% N found (calcd.)	4.75(4.88)	4.61(4.65)	3.74(3.71)	3.58(3.62)	3.84(3.88)	4.62(4.65)	4.33(4.35)	8.51(8.43)	8.48(8.43)	8.22(8.14)	9.71(9.79)	9.37(9.33)	7.50(7.44)	7.32(7.25)	7.74(7.77)	9.39(9.33)	8.96(8.86)	8.74(8.73)	8.78(8.73)	7.75(7.82)
% H found (calcd.)	6.39(6.32)	6.64(6.69)	6.49(6.41)	6.85(6.77)	6.73(6.69)	6.80(6.69)	5.39(5.33)	5.26(5.16)	5.22(5.16)	6.21(6.15)	6.74(6.69)	7.01(7.05)	6.78(6.69)	7.16(7.04)	7.06(6.99)	7.11(7.05)	6.62(6.69)	5.71(5.66)	5.69(5.66)	6.53(6.47)
% found (calcd.)	50.27(50.18)	51.43(51.83)	60.89(60.47)	52.50(52.71)	50.14(49.85)	51.64(51.83)	45.11(44.80)	43.29(43.38)	43.53(43.38)	48.72(48.84)	50.26(50.35)	51.87(52.00)	60.54(60.63)	52.69(52.85)	49.91(49.99)	51.79(52.00)	49.47(49.37)	44.85(44.94)	44.90(44.94)	50.16(50.28)
Molecular Formula (weight)	C ₁₂ H ₁₈ O ₅ NP(287.26)	C ₁₃ H ₂₀ O ₅ NP(301.28)	C ₁₉ H ₂₄ O ₅ NP(377.38)	C ₁₇ H ₂₆ O7NP(387.37)	C ₁₅ H ₂₄ O ₅ NSP(361.40)	C ₁₃ H ₂₀ O ₅ NP(301.28)	C ₁₂ H ₁₇ O ₅ NCIP(321.70)	C ₁₂ H ₁₇ O ₇ N ₂ P(332.25)	C ₁₂ H ₁₇ O ₇ N ₂ P(332.25)	C ₁₄ H ₂₁ O ₆ N ₂ P(344.31)	C ₁₂ H ₁₉ O ₄ N ₂ P(286.27)	C ₁₃ H ₂₁ O ₄ N ₂ P(300.30)	C ₁₉ H ₂₅ O ₄ N ₂ P(376.40)	C ₁₇ H ₂₇ O ₆ N ₂ P(386.39)	C ₁₅ H ₂₅ O ₄ N ₂ SP(360.42)	C ₁₃ H ₂₁ O ₄ N ₂ P(300.30)	C ₁₃ H ₂₁ O ₅ N ₂ P(316.30)	C ₁₂ H ₁₈ O ₄ N ₂ CIP(320.72)	C ₁₂ H ₁₈ O ₄ N ₂ CIP(320.72)	C ₁₅ H ₂₃ O ₆ N ₂ P(358.33)
D=0	1230	1230	1240	1230	1240	1250	1240	1230	1230	1240	1220	1220	1230	1220	1220	1230	1230	1220	1230	1230
N-C(Ar) or O-C(Ar)	1270	1270	1290	1270	1280	1270	1280	1270	1270	1280	1280	1280	1290	1280	1290	1290	1270	1280	1290	1280
P-N	970	980	980	970	980	086	066	980	970	970	980	975	980	970	980	980	970	980	960	980
P-OEt	1040	1040	1060	1060	1060	1030	1060	1060	1050	1060	1050	1050	1060	1050	1040	1030	1030	1050	1040	1030
C=C(Ar)	1600-1500	1605-1505	1600-1500	1600-1500	1610-1510	1600-1500	1600-1490	1610-1510	1600-1500	1610-1490	1605-1505	1600-1500	1600-1490	1590-1490	1605-1505	1600-1490	1600-1490	1605-1500	1610-1500	1620-1510
C=0	1730	1730	1740	1740	1750	1740	1730	1740	1730	1730	1740	1740	1730	1740	1750	1740	1750	1730	1745	1730
H-N	3230	3250	3300	3300	3300	3280	3200	3300	3150	3150	3200	3200	3200	3200	3190	3170	3160	3180	3200	3250
Com- pound	-	7	e	4	ŝ	9	7	80	6	10	Π	12	13	14	15	16	17	18	19	50

HUSSEIN M. ALI and ZIDAN H. ZIDAN

Company			Substituent	s on N		A connection of A = YD	Aromatic
compound	co-cu2-cu3 co-cu2-cu3	α CH or CH ₂	$\beta CH_2 or CH_3$	γ CH ₂ or Ph	8 SMe		substitution
-	4.10m(4H),1.25t(6H)	3.75m(2H)				7 20m(5H)	
7	4.10m(4H),1.20t(6H)	3.78m(H)	1.30d(3H)			7.20m(5H)	
3	4.20m(4H),1.30t(6H)	3.90m(H)	2.90m(2H)	7.24m(5H)*		7.24m(5H)*	
4	4.20m(6H),1.23m(9H)	3.75m(H)	2.27m(2H)	2.35t(2H)		7.15m(5H)	
5	4.15m(4H),1.20t(6H)	3.80m(H)	1.93m(2H)	2.50t(2H)	2.08s(3H)	7.22m(5H)	
9	4.10m(4H),1.28t(6H)	3.80m(2H)				7.35m(2H,0 to O),7.05m(2H)	2.30s(3H,Me)
7	4.17m(4H),1.20t(6H)	3.90m(2H)				7.20m(2H,0 to O),6.70m(2H)	
œ	4.08m(4H),1.35t(6H)	3.75m(2H)				7 25m(4H)	1.88s(3H,Me)
6	4.10q(2H)*,1.24t(3H)	4.00m(2H)*				7.40d(2H,0 to O),8.10d(2H)	
	4.40m(2H),1.32t(3H)						
10	4.10q(2H) [*] ,1.23t(3H)	4.00m(2H)*				8.12m(H,o to O/NO ₂),	
	4.40m(2H), 1.33t(3H)					7.20m(H,m to O),7.6m(2H)	
11	4.10m(4H),1.20t(6H)	3.65m(2H)				7.00m(5H)	
12	4.05m(4H),1.20t(6H)	3.70m(H)	1.30d(3H)			7.00m(5H)	
13	4.03m(4H),1.22t(6H)	3.70m(H)	2.90m(2H)	7.13m(5H)*		7.13m(5H)*	

TABLE II $^{\rm t}{\rm H}$ NMR data of phosphoramidates and phosphorodiamidates $^{\rm *}$

47

			Substituent.	s on N		A montain of A = VD	Aromatic
Compound		α CH or CH ₂	β CH ₂ or CH ₃	γ CH ₂ or Ph	в SMe	ry-12 fo minute	substitution
14	4.10m(6H),1.23m(9H)	3.71m(H)	2.29m(2H)	2.32t(2H)		7.05m(5H)	
15	4.12m(4H),1.20t(6H)	3.70m(H)	1.90m(2H)	2.50t(2H)	2.00s(3H)	7.10m(5H)	
16	4.05m(4H),1.20t(6H)	3.71m(2H)				7.05d(2H,o to N),6 80d(2H)	
17	4.10m(4H),1.18t(6H)	3.75m(2H)				7.20m(2H,0 to N),6.83m(2H)	
18	4.10m(4H),1.23t(6H)	3.68m(2H)				6.96m(4H)	2.25s(3H)
19	4.13m(4H),1.23t(6H)	3.70m(2H)				6.93d(2H,o to N),6.70d(2H)	2.70s(3H)
20	4.28m(4H)*,1.30t(6H)**	3.80m(2H)				6.94d(2H,0 to N),7.80d(2H)	4.28q(3H,Me) [*] , 1.3t(2H,CH ₂)**

* overlapped signals have similar signs.

PHOSPHORAMIDATES

Equations 1-4 reflected several features. First, All equations present strong correlations with a correlation coefficient (R) of 0.999, a standard deviation (s) < 0.018 and an F value > 99 indicating the reliability of these equations to predict the enzyme inhibition percentages by untested members of the two series. Table III includes the inhibition percentages calculated by equations 3 and 4. Second, equations 3 and 4 contain the same parameters with the same signs suggesting similar inhibition mechanisms in both series. Also, equations 3 and 4 have the same parameters present in equations 1 and 2, respectively, with the same signs and weighting factors. This indicates that changing R2 groups (the amino acid moiety) did not affect the inhibition mechanism in each series, but affected only the inhibitors' reactivity. Third, considering that bulky substituents have positive MR descriptors but negative Es descriptors, equations 3 and 4 showed that enzyme inhibition correlates inversely with the bulkiness of p-aryl substituents (R_1) and directly with the bulkiness of alkyl groups (R_2) . The absence of the MRm suggests that m-aryl substituents were directed away from AChE active site during the enzyme-inhibitor interaction. Previous results also showed that AChE inhibition was increased by increasing the bulk of alkyl groups of the amino acid moieties in some cyclic phosphorodiamidates, indicating the presence of a steric enzyme-inhibitor interaction during the inhibition process.²² Fourth, while the resonance parameter (R) showed that the inhibition process was enhanced by strong π -electron acceptor R₁ groups (e.g. NO₂), the field parameter (F) indicated increasing inhibition by strong electron donating groups. However, the weighting factor of the resonance parameter is ~ 2-fold that of the field parameter in all equations, which reflects its greater importance in determining the inhibition percentage. In addition, the inhibition process was inversely related to the electron donating ability of R₂ groups as presented by the polar parameter (σ^*). These results support the previously proposed inhibition mechanism of nucleophilic attack of a hydroxyl group in the enzyme active site on the partially positive phosphorus atom in organophosphorus compounds.^{22,23} Fifth, inhibition was increased by more hydrophobic substituents. It was reported that the hydrophobicity parameter (π) improved the correlation of anticholinesterase activity with the electronic parameters and played an important role in the inhibition process.²⁴ The above results showed the importance of both the reactivity of these compounds and their steric interaction with the AChE active site in controlling the enzyme inhibition as presented below.



Correlation between the observed %AChE inhibition and that calculated by equations 3 and 4 are presented graphically in Figure I. Table III also lists the median lethal concentrations LC_{50} of some compounds against mosquito larvae. The preliminary results showed that the phosphorodiamidates were much less toxic than the phosphoramidates which is consistent with previous results;¹³ however, quantitative description of this toxicity still requires additional research.

EXPERIMENTAL

All reagents and solvents were purified and dried according to the standard procedures. Amino acid esters were of L-(S)-configuration. IR spectra were recorded on Nicolet 460 FT-IR spectrometer. ¹H NMR spectra were performed on Varian EM 390 spectrometer by using CDCl₃as a solvent. Elemental analyses were carried out with a Perkin-Elmer 240 microanalyzer. GC-MS analyses were executed in electron impact mode on Finnigan Mat GCQ spectrometer equipped with Rtx-5MS column with 30 m long, 0.25 mm I.D. and 0.25 μ m film thickness (df). The oven temperature program was: initial value 40°C for 3 min then increased by 15°C/min till 250°C (5 min). Injection and detection temperatures were 150 and 250°C respectively.

Synthesis of phosphoramidates and phosphorodiamidates

The appropriate phenol or aniline (0.03 mol) and 3.12 g (0.03 mol) triethylamine in 20 mL dry THF were added dropwise on an ice-cooled solu-



FIGURE 1 Correlation between the observed %AChE inhibition and the calculated from equations 3 (phosphoramidate series) and 4 (phosphorodiamidate series)

tion of 5.0 g (0.03 mol) *O*-ethyl phosphorodichloridate in 30 mL dry benzene. The reaction mixture was stirred for two hours; then a mixture of 0.03 mol of an amino acid ester and 3.12 g triethylamine was added slowly while cooling at 0°C. The reaction mixture was stirred overnight then filtered, evaporated and dissolved in CH₂Cl₂. The phenolic products were washed with 20 mL 5 % NaOH while the anillinic products were washed with 20 mL 5% HCl. The crude product was chromatographed on silica gel column and eluted with C₆H₆/CH₂Cl₂ solvent system. Products were greasy. Yields were 55–65 % and purity, as checked by GC-MS, were > 97 %.

Anticholinesterase activity

Blood samples were freshly drawn from 3- to 6-month old Swiss white mice and centrifuged at 5000 rpm to collect the serum as enzyme source.

The clear serum was then frozen until used in the enzyme assay within two days. The assay was measured by the method of Ellman et al (1961).¹⁵ The inhibition percentage was determined in triplicate at 1 ppm concentration of each pesticide in the assay solution. The pesticide was added as a solution in ethanol.

	Phosphoramidates			Phosphorodiamidate	S
Com- pound	%AChE inhibition (obs)	(± SD) (calcd)	Com- pound	%AChE inhibition (obs)	(± SD) (calcd)
1	38.72 (± 0.86)	39.46	11	73.12 (± 1.15)	72.27
2	14.31 (± 0.57)	14.32	12	28.69 (± 0.71)	28.63
3	28.79 (± 0.73)	28.86	13	22.52 (± 0.64)	22.45
4	29.85 (± 0.79)		14	41.65 (± 0.83)	
5	28.84 (± 0.84)		15	25.66 (± 0.72)	
6	30.22 (± 0.75)	29.56	16	36.81 (± 0.78)	36.85
7	22.61 (± 0.66)	22.95	17	22.48 (± 0.59)	22.35
8	36.28 (± 0.90)	35.93	18	45.43 (± 0.88)	46.33
9	45.02 (± 1.03)	45.14	19	33.39 (± 0.80)	32.02
10	17.71 (± 0.63)	17.76	20	30.09 (± 0.74)	29.66
Com- pound	LC ₅₀ against mosquito larvae		Com- pound	LC ₅₀ against mosquito larvae	
1	31.83	-	- 11	131.77	-
3	25.75		12	194.23	
7	25.81		20	141.10	
8	56.52				

TABLE III Biological effects of O-ethyl phosphoramidate and phosphorodiamidates seies

Sumithion (0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate) inhbited AChE by 35% at the same concentration level (1 ppm).

Insecticidal activity against mosquito larvae

The larvicidal activity of the synthesized compounds were tested against mosquito larvae (*Cules pipiens* L.) by applying the WHO procedure

(1981).¹⁶ Each compound was dissolved in acetone in different concentrations. One mL of each concentration was pipetted into two beakers each containing 225 mL water (in duplicates). In the control experiment 1 mL of acetone was added. Lots of 20–25 mosquito larvae in 25 mL water was added to each beaker at 25 °C. After a period of 24 hours the mortality was counted.

Statistical analysis

A computerized multiple regression analysis was performed by stepwise introduction of a new parameter with minimizing the sum of squared deviations. The analysis was stopped when the correlation was no longer improved significantly as evidenced by the Student's *t* test. Descriptors of the stereo-electronic parameters^{17,18} and Hansch-Fujita's substituent parameters (π), characterizing hydrophobicity,¹⁹ used in the regression analysis were obtained from the literature. Model adequacy was measured by the correlation coefficient (*R*), the standard error (*s*) and the *F* value for analysis of variance at 95% confidence interval. The median lethal concentrations LC₅₀was computed by probit analysis for each compound.

References

- 1. R.J.W. Cremlyn, Pestic. Sci., 5, 667-73 (1974).
- 2. A.W. Garrison and C.E. Boozer, J. Am. Chem. Soc., 90, 3486-94 (1968).
- W.J. Stec, A. Okruszek, K. Lesiak, B. Uznanski and J. Michalski, J. Org. Chem., 41, 227-232 (1976).
- 4. R.-Y. Chen, H.-L. Wang & J. Zhou, Heteroatom Chem., 5, 497-501 (1994).
- 5. R.-Y. Chen and L.-J. Mao, Heteroatom Chem., 5, 125-29 (1994).
- 6. M. Aleksander and C. Krystyna, Pr. Inst. Przem. Org., 4, 131-42 (1972).
- 7. H. Kohsaka, Y. Oguri, M. Sasaki and K. Kukai, J. Pesticide Sci., 12, 415-19 (1987).
- 8. S. Giri and Y. Singh, Bokin Bobai, 6, 340-2 (1978).
- 9. S. Giri and Y. Singh, Bokin Bobai, 6, 301-4 (1978).
- 10. S. Giri and Y. Singh, Agric. Biol. Chem., 1275-8 (1977).
- 11. M. Ueji and C. Tomizawa, J. Pesticide Sci., 9, 675-80 (1984).
- 12. H. Yoshikawa, K. Fuchigami and T. Shono, J. Pesticide Sci., 11, 631-3 (1986).
- 13. H.M. Ali, Phosphorus, Sulfur and Silicon, in press.
- 14. H.M. Ali and A.A. Mostafa, Environ. Toxicol. Chem., 18, 167-71 (1999).
- G.L. Ellman, K.D. Courtney, K.D. Andres, Jr. and R.M. Featherstone, *Biochem. Pharmacol.*, 7, 88–95 (1961).
- WHO Instructions of determining the susceptibility or resistance of mosquito larvae to insecticides; WHO / VBC / 81.807; 1981.
- T.H. Lowry and K.S. Richardson, "Mechanism And Theory in Organic Chemistry" 3rd edn, Harper & Row Publishers, New York, 1987, pp. 143–158. And references therein.
- R.W. Taft, Jr., "Steric Effects in Organic Chemistry" MS Newman, John Wiley & Sons, Inc., New York, 1962.

- C. Hanch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry And Biology" John Wiley & Sons, Inc., New York, 1979.
- 20. S.M.M. Ismail, H.M. Ali and R.A. Habiba, J. Agric. Food Chem., 41, 610-5 (1993).
- 21. M. Ueji, and C. Tomizawa, J. Pesticide Sci., 9, 675-80 (1984).
- 22. H.M. Ali and K.A. Mohamed, Heteroatom Chem., in press.
- 23. K.A. Hassall, "The Biochemistry And Uses of Pesticides" 2nd ed., Macmillan Press Ltd., Hong Kong, 1990.
- T.R. Fukuto, R.L. Metcalf, R.L. Jones and R.O. Myers, J. Agric. Food Chem. 17, 923– 30 (1969).