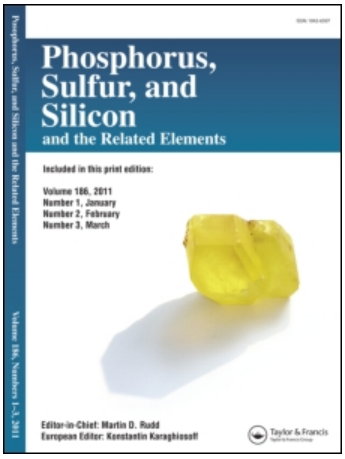

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SYNTHESIS AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF PHOSPHORAMIDATES AND PHOSPHORODIAMIDATES INCORPORATING AMINO ACID ESTERS

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Two series of *O*-aryl and *N*-aryl *O*-ethyl phosphoramidates and phosphorodiamidates respectively containing α-amino acid ester moieties have been synthesized and characterized by ^{1}H 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

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INTRODUCTION

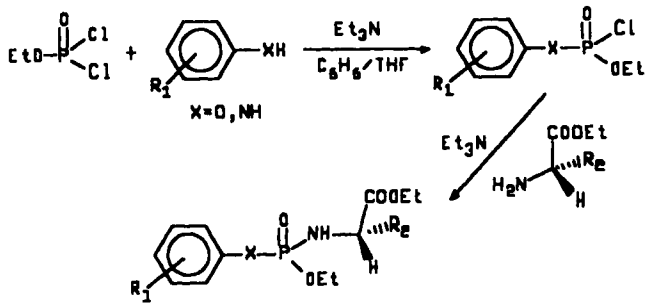
Phosphoramidates and phosphorodiamidates exhibited diverse biological activities. Their synthesis starts from phosphoryl chloride or its derivatives[1]. Their acid hydrolysis[2] and P-N bond cleavage[3] were investigated earlier. Incorporating amino acid ester derivatives[4] or a 1, 3, 5, 2-triazaphosphorine ring[5] 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-nitrophenyl[7] *N*-alkyl, *O*-4-methyl-7-hydroxycoumarin *N*, *N*-dialkyl and *O*, *O*-dialkyl[8] *N*-(β-phthalimidoalkyl)[9] or (sulfanilamide)[10] phosphoramidothioates exhibited some fungicidal activity. *O*-Ethyl *O*-2-isopropoxycarbonylphenyl *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.

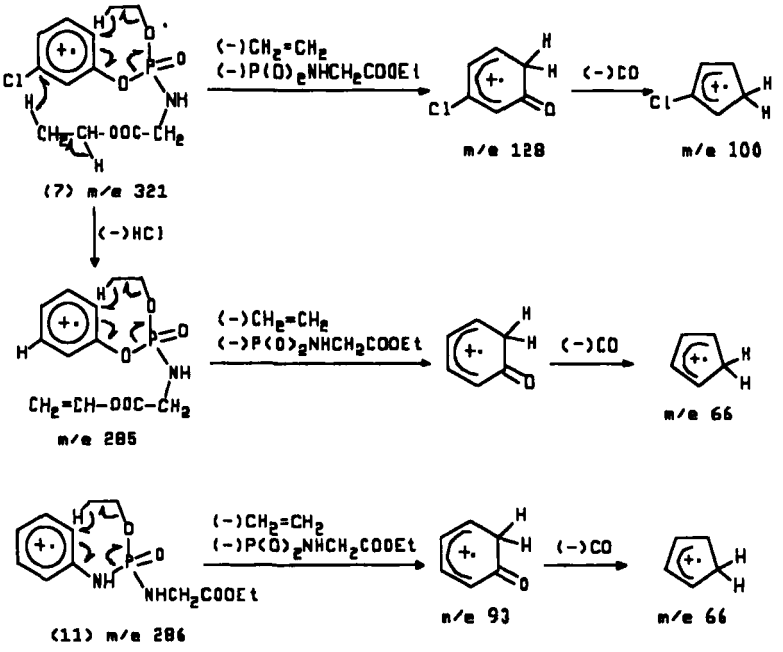
The characteristic IR bands of these compounds include $\nu_{P=O}$at ~ 1230, $\nu_{P\text{-}OEt}$ at ~ 1040, $\nu_{P\text{-}N}$ at ~ 970, $\nu_{PX\text{-}C(Ar)}$ at ~ 1280 and $\nu_{C=O}$ which varied from 1730–1750 cm^{-1}. This variation could be attributed to the participation in hydrogen bonding 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-



1 R_1 = H, X = O, R_2 = H (Gly)
2 R_1 = H, X = O, R_2 = CH_3 (Ala)
3 R_1 = H, X = O, R_2 = $PhCH_2$ (Phe)
4 R_1 = H, X = O, R_2 = EtOOC-CH_2-CH_2 (Glu)
5 R_1 = H, X = O, R_2 = $MeSCH_2CH_2$(Met)
6 R_1 = *m*-Me, X = O, R_2 = H (Gly)
7 R_1 = *m*-Cl, X = O, R_2 = H (Gly)
8 R_1 = *m*-NO_2, X = O, R_2 = (Gly)
9 R_1 = *p*-NO_2, X = O, R_2 = H (Gly)
10 R_1 = *p*-NHCOMe, X = O, R_2 = H (Gly)
11 R_1 = H, X = NH, R_2 = H (Gly)
12 R_1 = H, X = NH, R_2 = CH_3 (Ala)
13 R_1 = H, X = NH, R_2 = $PhCH_2$ (Phe)
14 R_1 = H, X = NH, R_2 = EtOOC-CH_2-CH_2 (Glu)
15 R_1 = H, X = NH, R_2 = $MeSCH_2CH_2$(Met)
16 R_1 = *p*-Me, X = NH, R_2 = H (Gly)
17 R_1 = *p*-OMe, X = NH, R_2 = H (Gly)
18 R_1 = *m*-CL, X = NH, R_2 = (Gly)
19 R_1 = *p*-Cl, X = NH, R_2 = H (Gly)
20 R_1 = *p*-COOEt, X = NH, R_2 = H (Gly)

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 ^{1}H 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



SCHEME I

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.[21] However, 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, R_1, were modeled by both the resonance parameter (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 MR*m*and MR*p*, 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.

$$\begin{aligned}&\text{Log\%AChE inhibition} = \qquad (1)\\ &1.612 + 0.194\text{R} - 0.226\pi - 0.015\ \text{MR}p - 0.104\text{F}\\ &(\pm 0.013)\,(\pm 0.011)\ (\pm 0.019)\quad (\pm 0.002)\quad (\pm 0.017)\\ &n = 6, R = 0.999, s = 0.015, F = 132.830\end{aligned}$$

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

$$\begin{aligned}&\text{Log\%AChE inhibition} = \qquad (2)\\ &1.884 - 0.023\ \text{MR}p + 0.189\text{R} - 0.199\pi - 0.072\text{F}\\ &(\pm 0.019)\quad (\pm 0.001)\quad (\pm 0.014)\ (\pm 0.034)\ (\pm 0.030)\\ &n = 6, R = 0.999, s = 0.018, F = 113.521\end{aligned}$$

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 (R_2) 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.

$$\begin{aligned}&\text{Log\%AChE inhibition} = \qquad (3)\\ &1.298 + 0.194\text{R} + 1.994\sigma^* - 0.535\text{Es} - 0.226\pi - 0.015\ \text{MR}p - 0.104\text{F}\\ &(\pm 0.019)(\pm 0.011)(\pm 0.099)\ (\pm 0.037)\ (\pm 0.019)\quad (\pm 0.002)\quad (\pm 0.017)\\ &n = 8, R = 0.999, s = 0.015, F = 157.275\end{aligned}$$

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

$$\begin{aligned}&\text{Log\%AChE inhibition} = \qquad (4)\\ &1.529 - 0.060\text{Es} - 0.023\ \text{MR}p + 0.189\text{R} - 0.072\text{F} - 0.199\pi + 0.745\sigma^*\\ &(\pm 0.027)(\pm 0.062)\quad (\pm 0.001)\quad (\pm 0.014)\ (\pm 0.030)\ (\pm 0.034)\ (\pm 0.152)\\ &n = 8, R = 0.999, s = 0.018, F = 99.633\end{aligned}$$

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.

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

Compound	*N-H*	*C=O*	*C=C(Ar)*	*P-OEt*	*P-N*	*N-C(Ar) or O-C(Ar)*	*P=O*	*Molecular Formula (weight)*	*% found (calcd.)*	*% H found (calcd.)*	*% N found (calcd.)*
1	3230	1730	1600–1500	1040	970	1270	1230	$C_{12}H_{18}O_5NP$(287.26)	50.27(50.18)	6.39(6.32)	4.75(4.88)
2	3250	1730	1605–1505	1040	980	1270	1230	$C_{13}H_{20}O_5NP$(301.28)	51.43(51.83)	6.64(6.69)	4.61(4.65)
3	3300	1740	1600–1500	1060	980	1290	1240	$C_{19}H_{24}O_5NP$(377.38)	60.89(60.47)	6.49(6.41)	3.74(3.71)
4	3300	1740	1600–1500	1060	970	1270	1230	$C_{17}H_{26}O_7NP$(387.37)	52.50(52.71)	6.85(6.77)	3.58(3.62)
5	3300	1750	1610–1510	1060	980	1280	1240	$C_{15}H_{24}O_5NSP$(361.40)	50.14(49.85)	6.73(6.69)	3.84(3.88)
6	3280	1740	1600–1500	1030	980	1270	1250	$C_{13}H_{20}O_5NP$(301.28)	51.64(51.83)	6.80(6.69)	4.62(4.65)
7	3200	1730	1600–1490	1060	990	1280	1240	$C_{12}H_{17}O_5NClP$(321.70)	45.11(44.80)	5.39(5.33)	4.33(4.35)
8	3300	1740	1610–1510	1060	980	1270	1230	$C_{12}H_{17}O_7N_2P$(332.25)	43.29(43.38)	5.26(5.16)	8.51(8.43)
9	3150	1730	1600–1500	1050	970	1270	1230	$C_{12}H_{17}O_7N_2P$(332.25)	43.53(43.38)	5.22(5.16)	8.48(8.43)
10	3150	1730	1610–1490	1060	970	1280	1240	$C_{14}H_{21}O_6N_2P$(344.31)	48.72(48.84)	6.21(6.15)	8.22(8.14)
11	3200	1740	1605–1505	1050	980	1280	1220	$C_{12}H_{19}O_4N_2P$(286.27)	50.26(50.35)	6.74(6.69)	9.71(9.79)
12	3200	1740	1600–1500	1050	975	1280	1220	$C_{13}H_{21}O_4N_2P$(300.30)	51.87(52.00)	7.01(7.05)	9.37(9.33)
13	3200	1730	1600–1490	1060	980	1290	1230	$C_{19}H_{25}O_4N_2P$(376.40)	60.54(60.63)	6.78(6.69)	7.50(7.44)
14	3200	1740	1590–1490	1050	970	1280	1220	$C_{17}H_{27}O_6N_2P$(386.39)	52.69(52.85)	7.16(7.04)	7.32(7.25)
15	3190	1750	1605–1505	1040	980	1290	1220	$C_{15}H_{25}O_4N_2SP$(360.42)	49.91(49.99)	7.06(6.99)	7.74(7.77)
16	3170	1740	1600–1490	1030	980	1290	1230	$C_{13}H_{21}O_4N_2P$(300.30)	51.79(52.00)	7.11(7.05)	9.39(9.33)
17	3160	1750	1600–1490	1030	970	1270	1230	$C_{13}H_{21}O_5N_2P$(316.30)	49.47(49.37)	6.62(6.69)	8.96(8.86)
18	3180	1730	1605–1500	1050	980	1280	1220	$C_{12}H_{18}O_4N_2ClP$(320.72)	44.85(44.94)	5.71(5.66)	8.74(8.73)
19	3200	1745	1610–1500	1040	960	1290	1230	$C_{12}H_{18}O_4N_2ClP$(320.72)	44.90(44.94)	5.69(5.66)	8.78(8.73)
20	3250	1730	1620–1510	1030	980	1280	1230	$C_{15}H_{23}O_6N_2P$(358.33)	50.16(50.28)	6.53(6.47)	7.75(7.82)

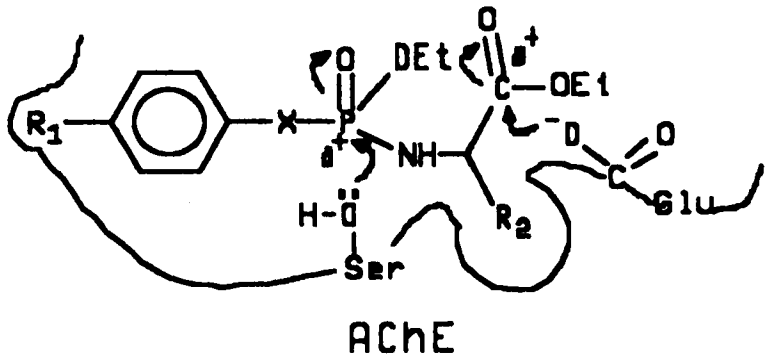
TABLE II ^{1}H NMR data of phosphoramidates and phosphorodiamidates*

Compound	*CO-CH_2-CH_3 PO-CH_2-CH_3*	*Substituents on N*				*Aromatic of Ar-XP*	*Aromatic substitution*
		α CH or CH_2	*β CH_2 or CH_3*	*γ CH_2 or Ph*	*δ SMe*		
1	4.10m(4H),1.25t(6H)	3.75m(2H)				7 20m(5H)	
2	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)	
6	4.10m(4H),1.28t(6H)	3.80m(2H)				7.35m(2H,*o* to O),7.05m(2H)	2.30s(3H,Me)
7	4.17m(4H),1.20t(6H)	3.90m(2H)				7.20m(2H,*o* to O),6.70m(2H)	
8	4.08m(4H),1.35t(6H)	3.75m(2H)				7 25m(4H)	1.88s(3H,Me)
9	4.10q(2H)*,1.24t(3H)	4.00m(2H)*				7.40d(2H,*o* 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)*	

Compound	*$CO\text{-}CH_2\text{-}CH_3$ $PO\text{-}CH_2\text{-}CH_3$*	*Substituents on N*				*Aromatic of Ar-XP*	*Aromatic substitution*
		α CH or CH_2	*β CH_2 or CH_3*	*γ CH_2 or Ph*	*δ SMe*		
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,*o* 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,*o* to N),7.80d(2H)	4.28q(3H,Me)*, 1.3t(2H,CH_2)**

* overlapped signals have similar signs.

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 R_2 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 MR*m* 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 R_1 groups (e.g. NO_2), 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 R_2 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 $CDCl_3$as 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 μm 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-

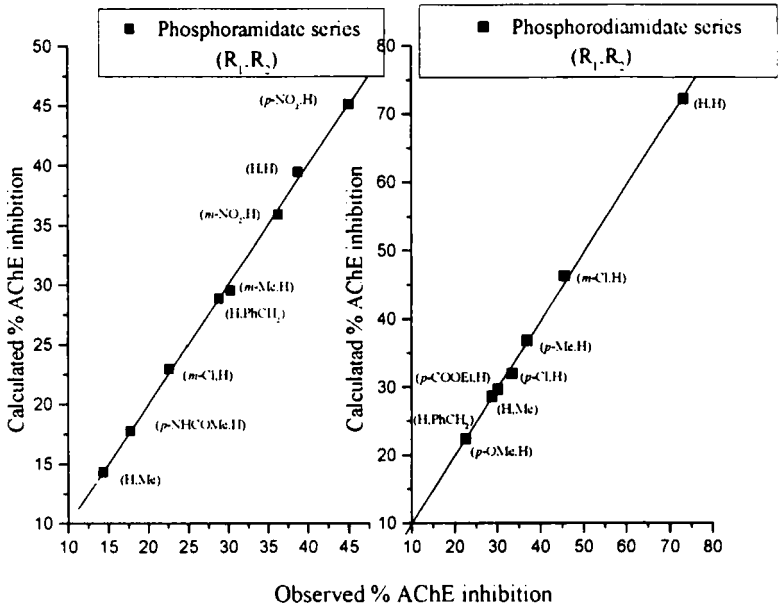


FIGURE 1 Correlation between the observed %AChE inhibition and the calculated from equations 3 (phosphoramidate series) and 4 (phosphorodiamidate series)

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 CH_2Cl_2. 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 C_6H_6/CH_2Cl_2 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.

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

Phosphoramidates			*Phosphorodiamidates*		
Compound	*%AChE inhibition (obs)*	*(± SD) (calcd)*	*Compound*	*%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

Compound	*LC_{50} against mosquito larvae*	*Compound*	*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		

Sumithion (*O,O*-dimethyl-*O*-(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 $LC_{50}$was computed by probit analysis for each compound.

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