Heteroatom Chemistry
Volume 10, Number 6, 1999

Synthesis and Bioactivity of *O*-Ethyl Phosphorodiamidates Derived from Quinazolin-4-ones and Either Amino Acid Esters or Fatty Amines

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Received 22 March 1999; revised 20 April 1999

ABSTRACT: *Synthesis of several O-ethyl phosphorodiamidates derived from unsubstituted, or 6-bromo-, or 6-nitro-3-amino-2-methyl-3H-quinazolin-4-one and either amino acid esters or fatty amines is described. These compounds showed high insecticidal activity toward mosquito larvae, with lethal concentrations LC_{50} and LC_{90} as low as 0.028 and 1.724 ppm, respectively. The highest activity was observed with those compounds containing both a nitro substituent and a 10-carbon-atom fatty-amine moiety. Multiple regression analysis was used to explain the larvicidal activity variation of these compounds. The larvicidal activity generally decreased according to the following order of amino acid moieties: glutamic acid > methionine > glycine > alanine > phenylalanine.* © 1999 John Wiley & Sons, Inc. Heteroatom Chem 10: 455–460, 1999

INTRODUCTION

Quinazoline ring system derivatives showed diverse biological activities. The synthesis and antimicrobial activity of some derivatives were achieved [1,2]. Quinazolines containing thioglycolic amino acid derivatives [3] or oxadiazolin-5-thione moieties [4] were prepared and used as antibacterial agents. Pharmacological activities of many quinazolones have been extensively studied [5–7]. In addition, some 4-quinazolineamines showed fungicidal activity [8]. In our research on the adverse effects of some organophosphorus pesticides on food and their structure-activity relationship [9–11], we found it interesting to prepare a series of phosphorodiamidates containing 3-amino-2-methyl-3*H*-quinazolin-4-one and either an amino acid ester or a fatty amine. Amino acid esters were incorporated in some drugs to lower their toxicity and improve the cellular uptake [12]. The fatty amine moiety could also enhance penetration of the insect through the cuticle. Preliminary tests indicate that these new phosphorodiamidates possess effective toxicity toward mosquito larvae. It was found that some formulated organophosphorus pesticides such as chlorpyrifos and sumithion achieved a mosquito larvae mortality level of 90–100% after a period of 48–85 days post-treatment [13]. It was also reported that 4-substituted 3,5-xylenyl diethyl phosphates [14], substituted acetophenone and benzaldehyde *O*-(diethyl

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 CCC 1042-7163/99/060455-06

phosphoryl) oxime [15], and some phosphoramidothionates and thiolates [16] have larvicidal activity (LC_{50}) toward mosquito larvae in the ranges 0.62–10, 0.02–10 and 0.7–10 ppm, respectively.

RESULTS AND DISCUSSION

Synthesis and Spectral Analysis

6-Unsubstituted, bromo or nitro-3-amino-2-methyl-3*H*-quinazolin-4-one derivatives (**4a–c**) were prepared by acetylation of methyl anthranilate with aqueous acetic anhydride to afford *N*-acetylanthranilate **2**, which was subjected to bromination or nitration to give methyl 5-bromo or nitro-acetamido benzoate **3a–b**. Cyclization of **2** or **3** with hydrazine in ethanol yielded the products **4a–c**. The phosphorodiamidates were then prepared by the reaction of the quinazoline amines (**4a–c**) with *O*-ethyl phosphorodichloridate in triethylamine as a base to give the phosphoramidic chlorides (**5**), which reacted either with amino acid esters or fatty amines to give compounds **6–14** or **15–22**, respectively, according to Scheme 1.

The IR spectra of the phosphorodiamidates were characterized by strong absorption bands of P–N near 950 cm^{-1}, P–O–Et at ~1060 cm^{-1} and P=O at ~1250 cm^{-1}. However, the latter was usually overlapped with the C–O (str). In addition, the lactam carbonyl absorbed at ~1680 cm^{-1}. The carboxylate carbonyl of amino acid derivatives (**6–14**) appeared at ~1740 cm^{-1}. The detailed IR information is listed in Table 1.

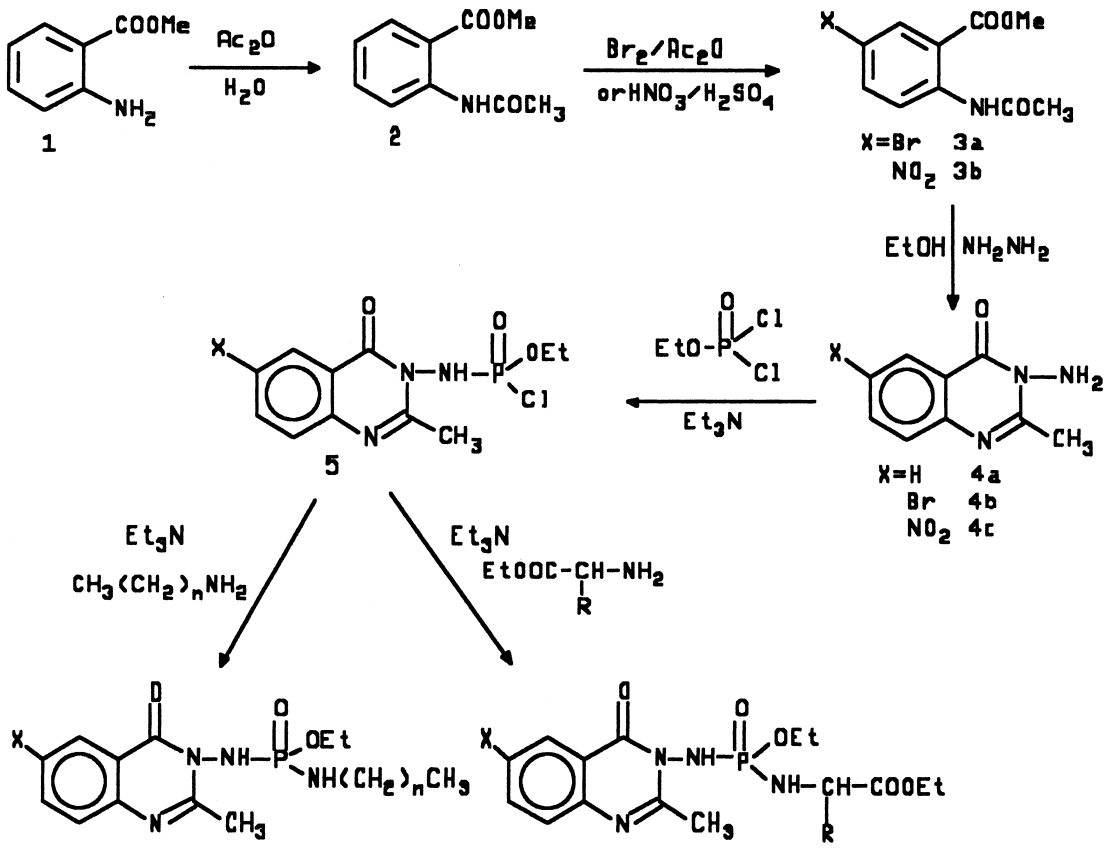
In the ^1H NMR spectra of these compounds, as presented in Table 2, the aromatic proton 5-H of the quinazoline ring gave a doublet of doublets at δ ~8.15 ppm with J_{ortho} ~9 Hz and J_{meta} ~2.7 Hz, while the two protons 6-H and 7-H and the proton 8-H gave multiplets at δ ~7.35 and ~7.6 ppm, respectively. In the 6-substituted ring, 5-H gave a doublet with a coupling constant J_{meta} ~2.7 Hz, and 8-H gave a doublet with a coupling constant J_{ortho} ~9 Hz. The signal of 7-H is split by 5-H and 8-H and thus gave a doublet of doublets with the mentioned coupling constants. In the amino acid ester derivatives (**6–14**), the proton signals of the two ethyl groups are overlapped. However, in fatty amine derivatives (**15–22**), the methylene proton signal of the *O*-ethyl phosphorodiamidates did not interfere with other signals and appeared as a doublet of quartets with coupling constants J_{H-P} ~20 Hz and J_{H-H} ~6 Hz. Other features of the spectra of the fatty amine derivatives are similar to those of the amino acid derivatives listed in Table 2 except for the presence of a multiplet of the N–CH_2 protons at δ ~2.9 ppm and a large multiplet at δ ~1.02 ppm for other aliphatic protons.

In mass spectrometry, the molecular ion peak of these high molecular weight phosphorodiamidates was either absent or quite small. Compound **19** gave M + 2 peak at *m/e* 502 because of the presence of a bromine atom. The base peak is usually due to a protonated fragment of one of the amine side chains; 3-amino-2-methyl-3 *H*-quinazolin-4-one (*m/e* 176) for **9** or decyl amine (*m/e* 158) for **19**. The common ions of *O*-ethyl phosphorodiamidates resulted from rearrangements that involve first a hydrogen migration to the lactam nitrogen with olefin formation and rupture of the N–N bond. The lost fragment gives an ion at *m/e* 239 in the case of **19**. Consecutive hydrogen migration with acetylene formation and loss of an alkyl group and hydrogen gave an ion at *m/e* 118 in the spectra of **9** and **19**. Also, cleavage of C–C bonds α-β and β-γ to the nitrogen of the amino acid with a double bond formation afforded an ion at *m/e* 147, which upon losing a hydrogen atom gave a peak at *m/e* 146 (spectrum of **9**). These ions are stabilized by the distribution of the charge on the two oxygen and two nitrogen atoms (Figure 1).

Larvicidal Activity

The lethal concentrations, LC_{50} and LC_{90}, of the synthesized phosphorodiamidates to mosquito larvae are presented in Table 3. Results showed that most compounds were effective larvicides. Their LC_{50} and LC_{90}, except for compounds **8**, **11**, **17**, **18**, and **21**, were less than 6.5 and 21, ppm respectively. The lowest lethal concentrations obtained were for compounds incorporating fatty amines with 10 or 12 carbon atoms (**15**, **16**, **19**, **20**, and **22**). The LC_{50} and LC_{90} for these compounds were less than 0.7 and 12.5 ppm, respectively, with those containing 10 carbon atoms being the most effective. Increasing the carbon chain in the fatty amine moieties and hence decreasing polarity of the compound increased the lethal concentrations, which indicates the importance of the polarity factor of the insecticide to penetrate the larvae. Also, nitro-substituted compounds (**13**, **14**, and **22**) were more effective than their bromo or unsubstituted analogs. Therefore, compound **22** containing 10-carbon-atom amine moiety and nitro substitution had the most larvicidal activity.

Phosphorodiamidates incorporating amino acid esters showed moderate larvicidal activity. The highest toxicity observed in this series was for compound **14** with a nitro substituent and glutamic acid moiety. Since it is difficult to explain the larvicidal activity variation of these phosphorodiamidates in terms of only one factor, a stepwise multiple regression analysis of compounds **6, 7, 8, 11,** and **13** was performed to study the effects of the aryl substituents and the



X = H, n = 9 **15**
H, 11 **16**
H, 13 **17**
H, 15 **18**
Br, 9 **19**
Br, 11 **20**
Br, 13 **21**
NO_2, 9 **22**

X = H, R = H (Gly) **6**
H, Me (Ala) **7**
H, Ph-CH_2 (Phe) **8**
H, EtOOC-CH_2-CH_2 (Glu) **9**
H, MeS-CH_2-CH_2 (Met) **10**
Br, Ph-CH_2 (Phe) **11**
Br, EtOOC-CH_2-CH_2 (Glu) **12**
NO_2, Ph-CH_2 (Phe) **13**
NO_2, EtOOC-CH_2-CH_2 (Glu) **14**

SCHEME 1

α-alkyl groups of amino acid moieties on the larvicidal activity. Regression analysis showed that log LC_{50} was strongly correlated with Taft's steric (*Es*) and polar (σ^*) factors of the α-alkyl groups and the field factor (*F*) of the aryl substituents as presented in equation 1:

$$\log LC_{50} = 0.783 - 0.276Es - 0.243F + 0.611\sigma^*$$
$$(\pm 0.021)\ (\pm 0.027)\ (\pm 0.028)\ (\pm 0.094)$$
$$n = 5,\ R = 0.996,\ s = 0.021,\ F = 38.159 \qquad (1)$$

where n = number of compounds, R = correlation coefficient, s = standard error, and F = significant index with respect to the equation at a 95% confidence interval. The calculated values of LC_{50} are included in Table 3. The absence of the resonance factor of the aryl substituents in the equation indicates that it has no effect on the larvicidal activity. Equation 1 explains 99% of the larvicidal activity variation. Results showed that the steric and polar factors of the α-alkyl groups and the field factor of aryl substituents contributed 29, 36, and 34% of these variations respectively. According to equation 1, the toxicity of the compound is directly proportional to the

TABLE 1 IR Absorption Bands of the Phosphorodiamidates (cm^{-1})

Compound	*NH*	*C=O (strz) of lactam-ester*	*P–N*	*P–O–E*	*C-O (str) or P=O*	*C=C (str)*	*Substituent*
6	3300	1680–1730	950	1065	1260	1610,1480	
7	3280	1670–1740	940	1060	1250	1610,1480	
8	3320	1680–1740	980	1050	1260	1600,1480	
9	3300	1685–1740	940	1050	1260	1600,1480	
10	3200	1680–1740	950	1060	1250	1600,1470	990,1300 (SMe)
11	3250	1680–1740	980	1050	1265	1605,1480	
12	3300	1690–1740	940	1050	1240	1600,1480	
13	3250	1680–1740	960	1060	1250	1600,1480	1350,1580 (NO_2)
14	3320	1680–1735	940	1050	1250	1605,1480	1345,1580 (NO_2)
15	3200	1680	960	1060	1250	1600,1475	
16	3420	1680	960	1050	1250	1605,1480	
17	3420	1680	960	1060	1250	1610,1485	
18	3420	1680	950	1070	1260	1605,1480	
19	3350	1680	950	1060	1240	1615,1480	
20	3200	1680	960	1060	1250	1620,1480	
21	3430	1680	940	1060	1250	1610,1475	
22	3200	1680	960	1060	1260	1605,1475	1350,1585 (NO_2)

TABLE 2 ^{1}H NMR Data of **4** and **6–14**[a]

	Quinazoline Ring Moiety						*Substituent on N*		
	5-H	*6,7-H*	*8-H*	*Me*	*NH*	*CO-$CH_2$$CH_3$[b] PO-$CH_2$$CH_3$*	α	β	γ or Ph
4a	8.12(H,dd)	7.33(2H,m)	7.60(H,m)	2.63(3H,s)	4.9				
4b	8.33(H,d)	7.77(H,dd)	7.57(H,d)	2.70(3H,s)	4.9				
4c	8,39(H,d)	7.82(H,dd)	7.62(H,d)	2.70(3H,s)	4.9				
6	8.10(H,dd)	7.30(2H,m)	7.57(H,m)	2.69(3H,s)	4.9	4.20(4H,m),1.23(6H,t)	3.75(2H,m)		
7	8.10(H,dd)	7.30(2H,m)	7.55(H,m)	2.69(3H,s)	4.5	4.10(4H,m),1.27(6H,t)[c]	3.64(H,m)	1.27(3H)[c]	
8	8.05(H,dd)	7.40(2H,m)	7.65(H,m)	2.68(3H,s)	4.8	4.97(4H,m),1.20(6H,t)	3.70(H,m)	3.08(2H,m)	7.20(5H,m)
9	8.17(H,dd)	7.40(2H,m)	7.62(H,m)	2.70(3H,s)	4.6	4.17(6H,m),1.27(9H,m)	3.90(H,m)	2.30(2H,m)	2.36(2H,m)
10	8.20(H,dd)	7.40(2H,m)	7.70(H,m)	2.67(3H,s)	5.3	4.10(4H,m),1.32(6H,t)	3.55(H,m)	1.95(2H,m)	2.56(2H,m)[d]
11	8.25(H,d)	7.80(H,dd)	7.15(H,d)	2.63(3H,s)	5.3	4.00(4H,m),1.18(6H,t)	3.70(H,m)	3.10(2H,m)	7.15(5H,m)
12	8.30(H,d)	7.90(H,dd)	7.22(H,d)	2.70(3H,s)	5.2	4.20(6H,m),1.30(9H,m)	3.70(H,m)	2.30(2H,m)	2.37(2H,m)
13	8.38(H,d)	7.80(H,dd)	7.61(H,d)	2.70(3H,s)	4.8	4.05(4H,m),1.20(9H,m)	3.72(H,m)	3.10(2H,m)	7.15(5H,m)
14	8.40(H,d)	7.90(H,dd)	7.64(H,d)	2.70(3H,s)	5.5	4.18(6H,m),1.32,(9H,m)	3.80(H,m)	2.23(2H,m)	2.33(2H,m)

[a]Chemical shifts in ppm.
[b]Signals of the ethyl groups are overlapped.
[c]Two interfered methyl signals.
[d]SMe: 2.10 (3H,s).

electron withdrawing ability of aryl substituents, which explains the high toxicity of compounds containing a nitro group. This field effect can be attributed to the enhancement of the electrophilicity of the neighboring carbonyl carbon that can participate in interaction with acetylcholinesterase enzyme of the mosquito larvae; it was reported by Fukuto et al. [15] that there is an excellent correlation between toxicity (LC_{50}) to mosquito larvae and anticholinesterase activity of some organophosphates and carbamoyloximes. Equation 1 also indicates that the toxicity of the compound is reduced by increasing the steric effect of α-alkyl groups of amino acid moieties and enhanced by the electron donating ability of the same groups. The larvicidal activity generally decreased according to the following order of amino acid moieties: glutamic acid > methionine > glycine > alanine > phenylalanine.

EXPERIMENTAL

Materials and Instrumentation

O-Ethyl phosphorodichloridate and *O*-ethyl-*N*-alkylphosphoramidic chloride **5** were manipulated under anhydrous conditions. Other reagents were reagent grade. NMR analysis was performed by using a Varian EM 390 NMR spectrometer and $CDCl_3$ as a solvent. GC-MS analysis was executed on a Finnigan

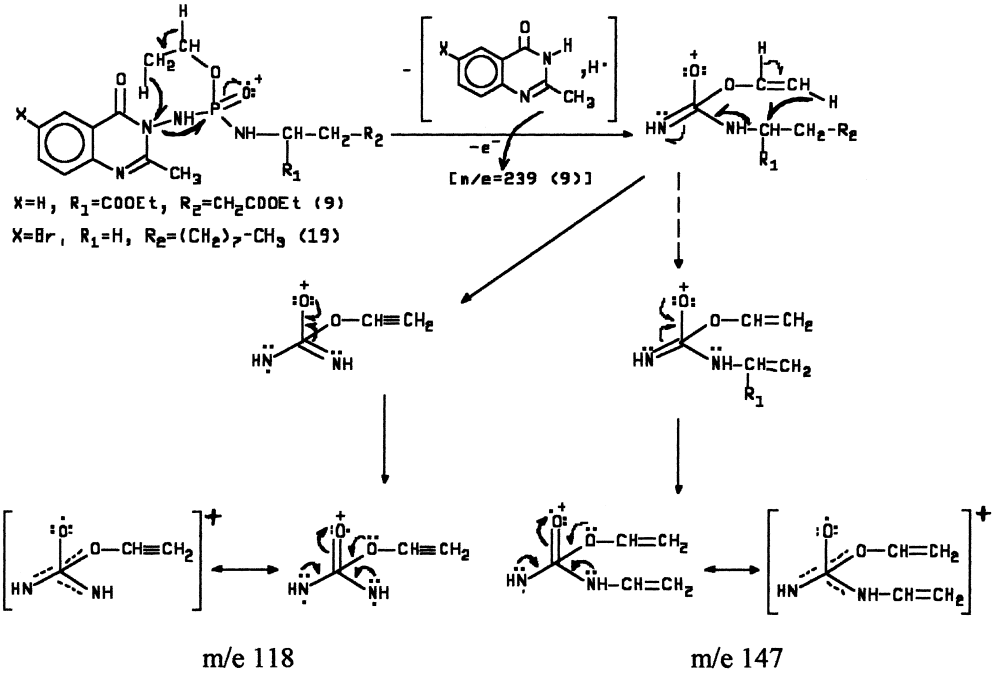


FIGURE 1 Common fragmentations and rearrangements of phosphorodiamidates **9** and **19** by mass spectrometry.

Mat system containing Tremetrics 9000 gas chromatograph equipped with a PTETMS fused silica capillary column (30 m, 25 mm i.d., and 0.25 μm film thickness) and an ion trap detector (ITD). Injection and detection temperatures were 250°C. The programming conditions were initial temperature at 70°C (1 min), increasing by 15°C/min to 150°C (5 min), followed by an increase of 25°C/min to a final temperature of 250°C (5 min). IR spectra were recorded on a Pye Unican SP3-100 grating spectrometer. Bromination and nitration of methyl *N*-acetylanthranilate **2** were effected by the general procedures [17].

Synthesis of Quinazolones **4**

To a solution of 20 g of the appropriate methyl *N*-acetylanthranilate **2** or **3** in 150 mL ethanol, 100 mL hydrazine hydrate was added, the solution was then refluxed for 2 hours. After cooling, 300 mL cold water was added with stirring. The crude product was collected by filtration and recrystallized from ethanol [18].

Synthesis of O-ethyl Phosphorodiamidates **6–22**

To a stirred solution of *O*-ethyl phosphorodichloridate (3.24 g, 0.02 mol) in 40 mL dry THF a solution of 0.02 mol of the appropriate amino quinazoline (**4a–c**) and 2.02 g (0.02 mol) of triethylamine in the same solvent was added dropwise while cooling at 0°C. Stirring was continued for 2 hours, then a mixture of 0.02 mol of amino acid ester or fatty amine and 2.02 g Et_3N in THF was added at 0°C. After stirring for 1 hour, the solvent was evaporated and the reaction mixture was dissolved in CH_2Cl_2 and washed with 5% NaOH, 5% HCl, and water. The organic phase was dried over $MgSO_4$ and evaporated. The oily product was purified by silica gel column chromatography with a hexane-CH_2Cl_2 solvent system. Yields ranged from 60 to 70% with purity >96% as indicated by GC–MS analysis.

Insecticidal Activity Against Mosquito Larvae

The WHO procedure was adopted [19]. Each compound (**6–22**) was dissolved in acetone in different concentrations. One milliliter of each concentration was pipetted into two beakers, each containing 225 mL water (in duplicate). In the control experiment, 1 mL of acetone was added. Lots of 20–25 mosquito larvae (*Cules pipiens* L.) in 25 mL water were added to each beaker at 25°C. The strain used had not been exposed to any chemicals since 1982. After a period of 24 hours, the mortality level was calculated and a computerized probit analysis was made to calculate the LC_{50} and LC_{90} for each insecticide.

Statistical Analysis

A computerized multiple regression analysis was performed by stepwise introduction of a new parameter, which minimized the sum of squared devia-

TABLE 3 The Observed and Calculated Lethal Concentrations of the Phosphorodiamidates to Mosquito Larvae (ppm)[a]

	Phosphorodiamidates Derived from					
	Amino Acid Esters				*Fatty Amines*	
	LC_{50}(*cal.*)	LC_{90}(*obs.*)	LC_{50}(*obs.*)		LC_{90}(*obs.*)	LC_{50}(*obs.*)
6	5.502	5.498	19.046	**15**	0.227	4.621
7	6.072	6.067	19.888	**16**	0.553	9.415
8	10.571	10.452	35.001	**17**	10.497	43.312
9	3.397	—	18.666	**18**	9.041	44.816
10	4.336	—	17.565	**19**	0.551	12.493
11	6.733	6.987	59.113	**20**	0.613	2.546
12	3.055	—	13.328	**21**	11.717	27.115
13	6.143	5.973	20.294	**22**	0.028	1.724
14	2.010	—	13.070			

[a]Each concentration was tested twice. The average number was used to calculate LC_{50} and LC_{90}.

tions. The analysis was stopped when the correlation was no longer improved significantly as shown by Student's *t*-test. The parameters used in the regression analysis were obtained from the literature [20]. Taft's steric and polar parameters of H, Me, and $PhCH_2$ groups are (1.24, 0, and -0.38) and (0.49, 0, and 0.215) respectively, while the field effect of the aryl substituents, H, Br, and NO_2, are 0, 0.72, and 1, respectively.

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