Journal of Enzyme Inhibition and Medicinal Chemistry, February 2006; 21(1): 105–111

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Anticholinesterase activity of some major intermediates in carbacylamidophosphate synthesis: Preparation, spectral characterization and inhibitory potency determination

KHODAYAR GHOLIVAND[1], AHLAM MADANI ALIZADEGAN[1], AZAM ANARAKI FIROOZ[1], KHOSRO KHAJEH[2], HOSSEIN NADERI-MANESH[2], & HAMIDREZA BIJANZADEH[2]

[1]*Department of Chemistry, Faculty of Science, Tarbiat Modarres University, P.O. Box: 14115-175, Tehran, Iran, and*
[2]*Department of Biochemistry and Biophysics, Faculty of Science, Tarbiat Modarres University, Tehran, Iran*

(Received 20 August 2005; in final form 12 October 2005)

Abstract
Carbacylamidophosphates with the general formula $RC(O)NHP(O)R_1R_2$ constitute organophosphorus compounds that are used as insecticides, pesticides and ureas inhibitors. In this work, we studied the inhibition potency of CCl_3-$C(O)NHP(O)Cl_2$**1**, $CHCl_2C(O)NHP(O)Cl_2$**2**, $CH_2ClC(O)NHP(O)Cl_2$**3** and $CF_3C(O)NHP(O)Cl_2$**4**, which are the major intermediates for carbacylamidophosphates synthesis towards human erythrocyte acetylcholinesterase (hAChe) activity using Ellman's modified kinetic method. Unexpectedly, it was observed that they were not only hydrolytically unstable but also inhibited hAChE in a similar manner to that produced by organophosphorus insecticides. Enzymatic data, bimolecular inhibition rate constants (k_i) and IC_{50} values for inhibition of hAChE demonstrated that they are irreversible inhibitors and the inhibition potency of compound **2** ($IC_{50} = 88\,\mu M$) was the greatest in comparison with compounds **1**, **3** and **4**. Also the electropositivity of the phosphorus atom and the hydrophobicity of the compounds demonstrated that these two factors play an additional effect and different role in the inhibitory activity of these compounds. Hydrolytic stability of the compounds was determined by ^{31}P NMR monitoring of the loss of the parent molecules with D_2O as a function of time. This study considers antiacetylcholinesterase activity according to the structural and the electronic aspects of compounds **1**–**4**, according to IR, ^{1}H, ^{13}C and ^{31}P NMR spectral data.

Keywords: *Carbacylamidophosphate, human erythrocytes acetylcholinesterase, hydrophobicity, IC_{50} value, irreversible inhibitor*

Introduction

The synthesis and biologically studies of organophosphorus compounds are of both practical relevance and academic interest [1,2]. The inhibitory potency of carbacylamidophosphates with the general formula $RC(O)NHP(O)R_1R_2$: which are a kind of organophosphorus derivatives, on urease activity has been studied [3]. *N*-haloacyl phosphoramidic dichlorides are the initial molecules which have been used for synthesis of many kinds of urease inhibitors and some other carbacylamidophosphate compounds. The existences of peptide and the phosphoryl group in such molecules leads to an interesting biological activity. It is known that the phosphoramidates with a P(O) moiety have an inhibitory effect on acetylcholinesterase (AChE) activity. The inhibitory potency of some reactants ($POCl_3$, PCl_3, and $PSCl_3$) in phosphoramidate production has been studied by Casida et. al. [4,5]. Their studies revealed that these compounds with a tendency for hydrolysis were AchE inhibitors. They also identified that phosphorodichloridic acid [$HOP(O)Cl_2$] acts as the actual phosphorylating agent of AChE in $POCl_3$. It seems that, such hydrolysis behavior may be observed in the *N*-haloacyl phosphoramidic dichloride. To extend and to develop, the synthesis, characterization, hydrolysis and the inhibition potency on human erythrocyte acetylcholinesterse activity of the compounds $RC(O)NHP(O)Cl_2$

Correspondence: K. Gholivand, Department of Chemistry, Faculty of Science, Tarbiat Modarres University, P.O. Box: 14115-175, Tehran, Iran. Tel: 98 21 88011001x3443. E-mail: gholi_kh@modares.ac.ir

ISSN 1475-6366 print/ISSN 1475-6374 online © 2006 Taylor & Francis
DOI: 10.1080/14756360500456764

with R = CCl_3 **1**, $CHCl_2$ **2**, CH_2Cl **3** and CF_3 **4**, are studied. Based on spectroscopic data and hydrophobicity evaluation (π_x), we considered the electronic properties of the phosphorus atom and the hydrophobicity of the surrounding substituents on the hydrolysis tendency and the inhibitory potency of the selected compounds.

Experimental

Materials and methods

All reactions in the synthesis of compounds from **1** to **4** were carried out under an argon atmosphere. Chemicals and enzymatic reagents were from the following sources: all of compounds and solvents for synthesis were from Merck, purified human plasma AChE (3.1.1.7) (50 units/785 μl) from Sigma, acetylthiocholine (ASCh), 5, 5′-dithio bis (2-nitrobenzoic acid) (DTNB) from Fluka. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker (Avance DRS) 500 MHz spectrometer and chemical shifts were obtained in $CDCl_3$ relative to TMS and 85% H_3PO_4, respectively, as external standards. IR spectra were obtained using KBr pellets on a Shimadzu IR-60 model spectrometer. Elemental analyses were performed using a Heraeus CHN-O-RAPID instrument and UV measurements were obtained with a Shimadzu UV-2100 spectrometer.

Chemical synthesis

All compounds **1–4** were synthesized according to reported methods [6,7], by reaction of (1.04 g, 5 mmol) PCl_5 in a solution of dry benzene and 2,2,2-trichloroacetamide (0.812 g, 5 mmol) for **1**, 2,2-dichloroacetamide (0.64 g, 5 mmol) for **2**, 2-chloroacetamide (0.47 g, 5 mmol) for **3**, and 2,2,2-trifluoroacetamide (0.565 g, 5 mmol) for **4**. The mixtures were refluxed for 4–18 h, then cooled to room temperature and treated with formic acid (0.23 g, 5 mmol) for 2 h at 15–20°C. The solvent was decanted leaving a white powder that was washed with dry benzene. Isolated yields for these compounds were between 90–98%. The results of spectral characterizations were as follows:

N-trichloroacetyl phosphoramidic dichloride [$CCl_3C(O)NHP(O)Cl2$] **1**. Anal. Calc. for $C_2HCl_5NO_2P$: C, 8.58; H, 0.3, N, 5.36; found: C, 8.6; H, 0.4; N, 5.29%; ^1H NMR ($CDCl_3$), δ (ppm): 9.94(s, 1H, NH); ^{13}C NMR($CDCl_3$), δ (ppm): 91.00(d., CCl_3, $^3J_{PC}$ = 13.1 Hz), 160.3(d, C(O), $^2J_{PC}$ = 2.8 Hz); ^{31}P {^1H} NMR($CDCl_3$), δ (ppm): 8.08(s); IR(KBr), ν (cm^{-1}): 408(w), 521(m), 590(s), 665(vs), 817(s), 902(s), 1181(s), 1274(vs), 1426(vs), 1736(s), 2835(w), 3045(m).

N-2,2-dichloroacetyl phosphoramidic dichloride [$CHCl_2C(O)NHP(O)Cl_2$] **2**. Anal. Calc. for $C_2H_2Cl_4NO_2P$: C, 9.79; H, 0.81; N, 5.71; found: C, 9.81; H, 0.79; N, 5.69%; ^1H NMR($CDCl_3$), δ (ppm): 9.68(d, 1H, NH, $^2J_{PNH}$ = 10.5 Hz), 5.98(1H, d, $^3J_{P\text{-}CH}$ = 2.3 Hz); ^{13}C NMR($CDCl_3$), δ (ppm): 65.67(d, $CHCl_2$, $^3J_{PC}$ = 12.0 Hz), 163.24(d, CO, $^2J_{PC}$ = 3.6 Hz); ^{31}P {^1H} NMR($CDCl_3$), δ (ppm): 8.2(s); ^{31}P NMR($CDCl_3$), δ (ppm): 8.2 ((dd), 12.0 Hz); IR(KBr), ν (cm^{-1}): 502(w), 580(m), 663(m), 764(w), 805(w), 885(m), 952(w), 1194(m), 1280(s), 1421(vs), 1710(vs), 2830(w), 3050(m).

N-2-chloroacetyl phosphoramidic dichloride [$CH_2ClC(O)NHP(O)Cl_2$] **3**. Anal. Calc. for $C_2H_3Cl_3NO_2P$: C, 11.40; H, 1.42; N, 6.65; found: C, 11.18; H, 1.50; N, 6.71%; ^1H NMR($CDCl_3$), δ (ppm): 9.16(d, NH, $^2J_{PNH}$ = 11.6 Hz), 4.16(d, 2H, CH_2Cl, $^3J_{PCH}$ = 1.2 Hz); ^{13}C NMR($CDCl_3$), δ (ppm): 43.65(d, CH_2Cl, $^3J_{PC}$ = 10.5 Hz), 167.12(d, CO, $^2J_{PC}$ = 3.8 Hz); ^{31}P {^1H} NMR($CDCl_3$), δ (ppm): 6.62(s); ^{31}P NMR($CDCl_3$), δ (ppm): 6.62((dd), 13.2 Hz); IR(KBr), ν (cm^{-1}): 531(w), 585(s), 875(m), 943(m), 1125(s), 1260(s), 1451(vs), 1721(vs), 2940(m), 32059(s).

N-2,2,2-triflouroacetyl phosphoramidic dichloride [$CF_3C(O)NHP(O)Cl2$] **4**. Anal. Calc. for $C_2HCl_2F_3NO_2P$: C, 10.43; H, 0.43; N, 6.08; found: C, 9.98; H, 0.51; N, 7.02%; ^1H NMR ($CDCl_3$), δ (ppm): 10.24(s, NH); ^{13}C NMR ($CDCl_3$), δ (ppm): 115.4((qd), CF_3, $^1J_{CF}$ = 288.5 Hz, $^3J_{CP}$ = 16.4 Hz); 157.5((qd), CO, $^2J_{CF}$ = 45.8 Hz, $^2J_{CP}$ = 3.5 Hz); ^{31}P {^1H} NMR ($CDCl_3$), δ (ppm): 7.42(s); IR (KBr), ν (cm^{-1}): 515(w), 593(m), 878(m), 1131(s), 1180(vs), 1211(m), 1279(m), 1311(w), 1466(m), 1748(m), 2900(w), 3075(m).

AChE activity assay

The activity of hAChE was determined by a modified Ellman's method [8], using the level of ASCh hydrolysis by monitoring thiocholine liberation with DTNB reagents. Reaction was carried out at 25°C in 70 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH = 7.4, 920 μl volume) containing the enzyme (15 μl volume, diluted 100 times in phosphate buffer, pH = 7.4), DTNB (10^{-4} M final concentration, 50 μl volume) and ASCh (1.35×10^{-4} M final concentration, 15 μl volume). The absorbance change was monitored at 418 nm for 2 min, and five replicates were run in each experiment. In the absence of inhibitor, the absorbance change was directly proportional to the enzyme level.

AChE inhibition experiments. The reaction mixtures for determination of IC_{50} values, the median inhibitory concentration, consisted DTNB solution, 50 μl; inhibitor, x μl (5–200); acetylthiocholine (ASCh) solution, 15 μl; phosphate buffer (920 − x) μl; hAChE solution, 15 μl. The final concentrations of DTNB, ASCh, and inhibitors from **1**–**4** were: 10^{-4}, 1.35×10^{-4}, ($2.16 \times 10^{-3} - 15.13 \times 10^{-3}$ M), ($3.3 \times 10^{-5} - 2.3 \times 10^{-4}$ M), ($9 \times 10^{-5} - 1.03 \times 10^{-3}$ M), ($4.7 \times 10^{-4} - 4.1 \times 10^{-3}$ M), respectively. The enzyme concentration in the assay was 2.51×10^{-10} M. In the four inhibitor solutions in phosphate buffer, 5 μl of dry THF was added as carrier solvent. The THF carrier (up to 5 μl) per assay did not affect the AChE activity [4]. The reaction mixtures for determination of the inhibition mechanism and k_i values were: DTNB and ASCh (the same as above); a solution of enzyme plus inhibitors (inhibitor concentration was adjusted to give about 50% of hAChE inhibition) 100 μl; phosphate buffer, 835 μl.

Determination of enzyme activity after incubation of inhibitors in phosphate buffer for 40 min. Enzyme activity determinations after 40 min incubation of inhibitors **1**, **2**, **3** and **4** in phosphate buffer (pH = 7.4) were performed for recognizing the active species that reacted with hAChE via their hydrolysis activity. It was found that at 40 min all four compounds were hydrolyzed completely and the chlorine atoms replaced by OH groups. After this time, no change in enzyme activity in the presence of the inhibitors was shown, during the enzyme activity assay (the reaction mixtures were in accordance with the section on AchE inhibition experiments).

Hydrolysis study of $RC(O)NHP(O)Cl_2$ compounds and stability of intermediates

Hydrolytic activation and products. Hydrolytic activation of compounds **1**–**4** was determined by loss of the parent compounds in D_2O as a function of time at 25°C. After five min. for scanning ^{31}P NMR spectra, two major peaks were observed. The chemical shifts were as follows: Compound, $^{31}P\{^1H\}$ NMR, δ (ppm): **1**, −5.76(s), −11.44(s); **3**, −5.59(s), −17.53(s); **4**, −6.93(s), −19.6(s). The intensity ratio of the two major peaks was relatively 5:1 for each compound. However, despite these results, only one single peak in the ^{31}P NMR spectra of compound **2** in D_2O was observed at $\delta = -5.26$ ppm which may correspond to the dihydroxylated derivative. A single peak at $\delta = 0$–0.2 ppm with medium intensity was observed, corresponding to phosphate. The second peak in compounds **1**, **3** and **4** (−11.44, −17.53 and −19.6 ppm) gradually disappeared after 30, 15 and 10 min respectively. It is proposed that the most intense peaks at $\delta = -11$ to -19 ppm were related to monohydroxylated substituents, which were not stable and converted to dihydroxylated substituents at different times. Synthesis of dihydroxylated derivatives of compounds **1**–**4** confirmed our suggestion.

Synthesis of $RC(O)NHP(O)(OH)_2$. A solution of water (6.6 mmol) in acetone (10 ml) was added dropwise to each of compounds **1**–**4** (3.3 mmol) in dry acetone (10 ml) while stirring at 25°C. The reaction mixtures were stirred for 20 min. ^{31}P NMR (10% C_6D_6 in acetone) spectra for the synthesized compounds showed the following major peaks: Compound, $^{31}P\{^1H\}$ NMR, δ (ppm): **1**, −5.26(s); **2**, −5.33(s); **3**, −5.48(s); **4**, −6.2(s). These major peaks corresponded to $RC(O)NHP(O)(OH)_2$ where $R = CCl_3$, $CHCl_2$, CH_2Cl, CF_3. All of the compounds are stable in acetone.

Hydrophobic parameter evaluation

The octanol–water partition coefficient, log ($P_{o/w}$), for compounds **1**–**4** were calculated according to Leo et al. [9]. The following equation: $\pi_x = \log P_x - \log P_H$ was used for determining the hydrophobicity extent of substituents around the phosphorus atom, where P_x is the derivative of a parent molecule, P_H, and thus π_x is the hydrophobicity of the function x. Because the $P(O)Cl_2$ group was constant in all four compounds, we concentrated on the RC(O)NH moiety for calculating log ($P_{o/w}$), in comparison with acetamide as the parent molecule. By this manner, the additional hydrophobicity of chlorine atoms in the terminal haloacyl group for compounds from **1**–**3** and fluorine atoms in compound **4** were evaluated. The π_x values, shown in Table I, indicated that molecule **1** with three chlorine atoms has the most hydrophobicity.

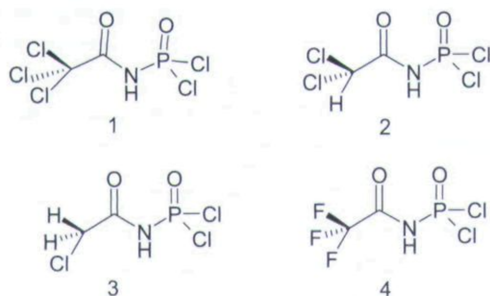
Relationship between the phosphorylation rate constants and phosphorus-31 NMR chemical shifts

By comparing the inhibition rate constants (k_i) and the ^{31}P NMR chemical shifts for compounds **1**–**4**, no relationship was observed between these two factors for these molecules.

Results

Synthesis and spectral data

The synthesis of compounds **1**–**4** has been previously reported and characterized by IR and elemental analysis [3,6,7,10–12] and the compound **4** was additionally characterized by IR, ^1H, ^{13}C and ^{19}F NMR spectroscopy [13]. In this work, more characterization **1**–**4** has been achieved by IR, ^1H, ^{13}C, ^{31}P NMR spectroscopy and elemental analysis. (Scheme 1)



Scheme 1. Structures of compounds **1–4**.

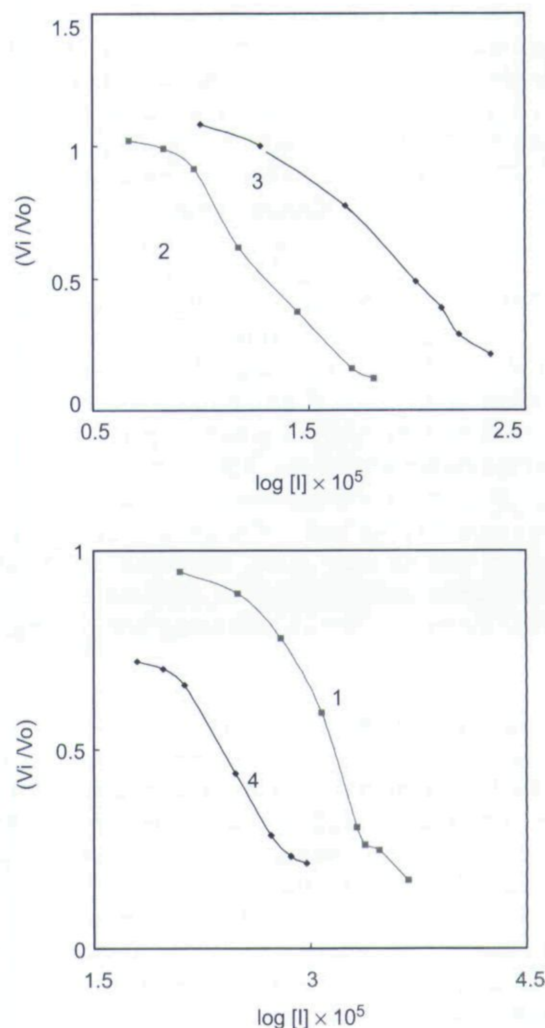


Figure 1. The plot of V_i/V_o against Log [I] × 10^5 for inhibitor from **1** to **4**: V_i and V_o are activity of enzyme in the presence and absence of inhibitor, respectively and [I] is inhibitor concentration (mol/lit), *means ± SE ($n = 4$).

^{1}H NMR spectroscopic data for the compounds **2** and **3** indicate two-bonds coupling between phosphorus and the NH hydrogen atom as $^2J_{PNH} = 10.5$, 11.6 Hz, respectively, where these values are in agreement with other phosphoramidate compounds [14]. This coupling not present in compounds **1** and **4**. Also in compounds **2** and **3**, the coupling between phosphorus and the hydrogen atom of the terminal haloalkyl was observed as $^3J_{PCH} = 2.3$, 1.2 Hz, respectively. ^{13}C NMR spectra showed the coupling between the phosphorus atom and the carbonyl carbon atom as $^2J_{PC} = 2.8$ Hz **1**, 3.6 Hz **2**, 3.8 Hz **3** and 3.5 Hz **4**. Three bonds coupling between phosphorus and the haloalkyl carbon atom, which were obtained by ^{13}C NMR spectra, are 13.1 Hz **1**, 12.0 Hz **2**, 10.5 Hz **3**. In the case of compound **4**, two different couplings, as a doublet of quartet, which arise from the fluorine and phosphorus atom, were observed in the ^{13}C NMR spectra of CF_3 group, ($^1J_{FC} = 288.5$ Hz and $^3J_{PC} = 16.4$ Hz). A similar effect was observed for the carbonyl carbon atom with $^2J_{FC} = 45.8$ Hz and $^2J_{PC} = 3.5$ Hz, which it leads to another doublet of quartet in ^{13}C NMR spectra. These data were consistent with a previous report [13]. Also the infrared spectral data and the elemental analys for all four compounds corresponded to other previous reports [11,12].

Human acetylcholinesterase inhibition

The inhibitory effect of 6 different concentrations of each of the carbacylamidophosphates **1–4** hAChE activity was determined. The IC_{50} values of these four compounds derived from Figure 1, are given in Table I. Figure 2, shows the typical time-course for the inhibition of human erythrocyte AChE by compounds **1–4**, plotted as (ln remaining enzyme activity (V_i/V_o)) vs time. The inhibition rate constants for compounds **1–4**, K_i, under pseudo-first-order conditions were computed from the slopes of the plots in Figure 2 [15,16] and the values are given in Table I. The plots in Figure 2 gave intercepts at $V_i/V_o < 1$ and the K_i values, dissociation equilibrium constants, were calculated from the equation $V_i/V_o = (1 + s_o/K_m)/(1 + s_o/K_m + i/K_i)$ for the compounds (Table I).

Also the K_m and V_{max} values of hAChE under the experimental conditions used were obtained as 10.820×10^{-4} mol L^{-1} and 10.03×10^{-6} mol L^{-1} min^{-1}, respectively [17]. The values show that, the compound **2** has the largest phosphorylation rate constant ($k_i = 0.31$ min^{-1}) and the smallest dissociation equilibrium constant ($k_i = 0.036$ mM) in comparison with the other molecules. It demonstrates that the formation of the phosphoAChE association between this molecule and the enzyme is the most effective, which leads to more inactivation of AChE activity.

Discussion

The ^{1}H NMR spectra of the synthesized compounds present useful information about the coupling of the amidic proton with the phosphorus atom ($^2J_{PNH}$). As shown in Table I the coupling constant in **2** is $^2J_{PNH} = 10.5$ Hz and it for **3** is $^2J_{PNH} = 11.5$ Hz.

Table I. The spectral and enzymatic data for compounds **1**–**4** where R is[C(O) NHP(O)Cl2].

Compound	$\nu_{P(O)}$ cm^{-1}	$\nu_{C(O)}$ cm^{-1}	ν_{P-Cl} cm^{-1}	δ^{31}_{P} ppm	IC_{50*}µM	$\log(P_{o/w})$	π_x	k_i (min^{-1})	K_i mM
CCl_3R **1**	1181	1736	590	8.08	5280 ± 120	−0.09	1.17	0.056	9.74
$CHCl_2R$ **2**	1194	1710	580	8.20	88 ± 22	−0.48	0.78	0.31	0.036
CH_2ClR **3**	1125	1721	585	6.62	432 ± 35	−0.87	0.39	0.169	0.206
CF_3R **4**	1180	1748	593	7.42	1030 ± 24	−1.77	−0.51	0.058	0.376

Unexspectedly, these couplings were absent in the **1** and **4**. Electronwithdrawing effect of the chlorine and the fluorine atoms in **1** and **4** may be increasing the acidity of NH proton (δ = 9.94 ppm for **1** and 10.26 ppm for **4**) and this may account for the absence of $^2J_{PNH}$. The inhibition potency of the compounds indicates an increasing inhibitory effect on hAChE: **2** > **3** > **4** > **1**. as obtained by IC_{50} values comparison. The inhibition potency of most organophosphorus compounds is dependent on the leaving group, the charge on the phosphorus atom and the phosphorus atom substitutions [18]. The studies on these parameters is described as follows:

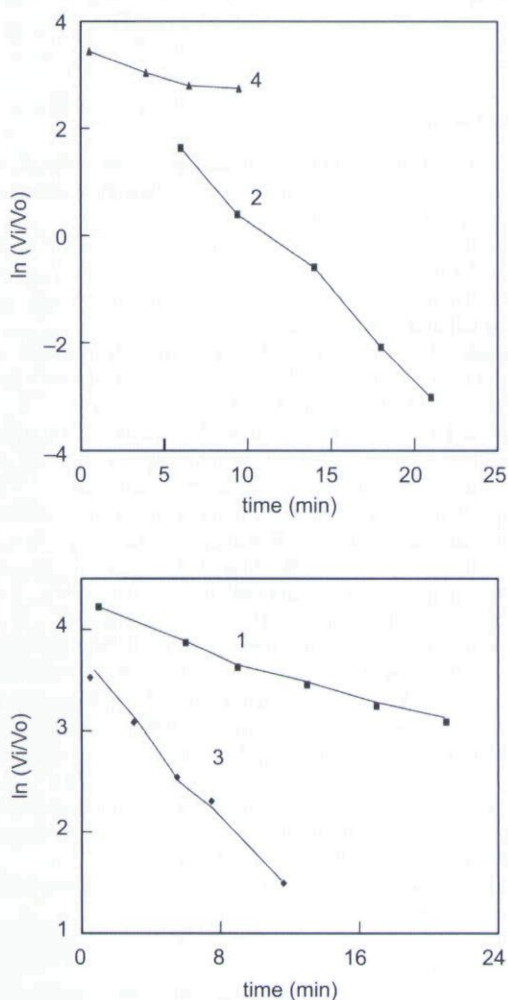


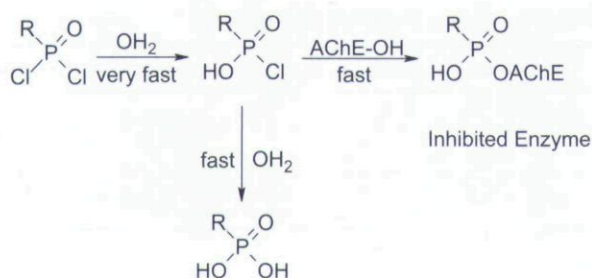
Figure 2. Plots of ln (V_i/V_o) versus time (min) for inhibitors **1**–**4**.

Leaving group

The mechanism of acetylcholinesterse inactivation by organophosphorus compounds occurs with the ejection of a leaving group, synchronous with formation of a phosphoserine linkage [19,20]. In all four compounds, the chlorine atom of the P–Cl bond is the best leaving group. A decreasing in bonding energy of P–Cl makes it more labile and it leads to either a better interaction with the enzyme active site and or faster displacement with an OH group of aqueous solution. As shown in Table I, compound **2** has the smaller ν_{P-Cl} in comparison with the other compounds which leads to it being a more potent inhibitor of AchE. Also, it tends to hydrolyses faster than the others in aqueous solution. Mono- and dihydroxylated compounds from **1** to **4** are formed by replacing the chlorine atom with the OH group during the hydrolysis via bimolecular mechanism[22]. The dihydroxylated compounds have no inhibitory potency on hAChE, because the OH group is a weak leaving group. This is demonstrated by determining enzyme activity in the presence of these completely hydroxylated inhibitors (after 40 min incubation in phosphate buffer). These experiments shows no change in enzyme activity and reveal that dihydroxylated compounds, $RC(O)NHP(O)(OH)_2$, are not AChE inhibitors. Therefore, the inhibition potency and the hydrolysis tendency of compounds from **1**–**4**, establishes that the monohydroxylated species, $RC(O)NHP(O)(OH)Cl$, have hAChE inhibition potency. The result compare with compound $OHP(O)Cl_2$, which is the hydrolyzed species of $POCl_3$, that can act as an AChE inhibitor with IC_{50} = 12–36 µM [4]. The proposed mechanism for hAChE inhibition by **1**–**4** following hydrolytic activation to hydroxylated species is presented in Scheme 2.

Charge on the phosphorus atom

Electropositivity of the phosphorus atom probably is the reason for better linkage of the OH-serine group with the P(O) moiety. ^{31}P NMR spectra for these compounds showed that the decreasing order of the chemical shifts are as follows: **2** (δ = 8.2 ppm) < **1** (δ = 8.08 ppm) < **4** (δ = 7.42 ppm) < **3** (δ = 6.62 ppm)



Scheme 2. Proposed mechanism for hAChE inhibition by compounds **1**, **2**, **3** and **4** following hydrolytic activation to RP(O)(OH)Cl, where R = CCl_3**1**, $CHCl_2$ **2**, CH_2Cl **3**, CF_3 **4**.

Compound **2** has the largest amount of chemical shift as shown in Table I. It might be expected that having the most electropositive phosphorus atom perhaps is an important factor that makes it the strongest inhibitor ($IC_{50} = 88\ \mu M$). Surprisingly, compound **3**, which has the lowest chemical shift, is a more potent inhibitor than compounds **1** and **4** due to less hydrophobicity of the substituents around the phosphorus atom.

The phosphorus atom substituents

The reaction of AChE with organophosphorus compounds displays marked stereoselectivity [21], which could be utilized for an investigation of the hydrophobic and the steric interactions with the structural elements of the active center. AChE substrates or their equivalents (inhibitors such as the organophosphorus compounds) fit into a crevice which tends to exclude larger molecules [25,26], and also limits hydrophobic binding of large substituents, hence their absence in acetylcholine, which is the normal substrate of AChE. By calculating log ($P_{o/w}$) and π_x, the hydrophobicity of the compounds can be evaluated. The increasing order in hydrophobic potency according to the data in Table I is **1** > **2** > **3** > **4**.

By considering the fact that hydrophobic substituents hinder, rather than enhance the association of organophosphorus compounds with AChE [23,24], unexpectedly molecule **2** with more hydrophobicity relative to the other compounds (except **1**), has the most inhibitory potency. It is proposed that this can be related to the greater electropositivity of the phosphorus atom in this molecule ($\delta^{31}P$ NMR = 8.2 ppm), which leads to rapid nucleophilic substitution independently of the hydrophobicity of the substituents around the phosphorus atom. Structural comparison of compounds **1** and **2** shows that by replacement of a chlorine atom with hydrogen, despite a negligible difference in ^{31}P NMR chemical shift, compound **2** is 60-fold stronger inhibitor than compound **1**. Thus might be relative to the difference between the hydrophobicity of the two molecules, as the log *P* for compound **1** is 5 fold greater than that for compound **2**.

Conclusion

This investigation establishes that the acute toxicity of the compounds on hAChE could be attributable to hydrolytic activation of RC(O)NHP(O)(OH)Cl which then phosphorylates AChE to form the enzymatically inactive phosphoAChE. The data reinforce the reported importance of the hydrophobicity of the substituents and the electropositivity on the phosphorus atom as important determinants of the inhibitory potency of organophosphorus compounds on AChE. The data reveals that the two factors mentioned above, have a different share of the inhibitory potency of each compound. It is proposed that where the ^{31}P NMR chemical shift determining the electropositivity of specified compounds have small differences, the hydrophobic parameter of the substituents is an important factor for the differences in the inhibitory effect. Inversely, the differences in the electropositivity of the phosphorus atom might be the effective factor for the compounds with small differences in the hydrophobicity of their substituents.

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