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Preliminary Communication

Synthesis of novel phosphorothioates and phosphorodithioates and their differential inhibition of cholinesterases

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ABSTRACT

The anticholinesterase activities of newly synthesized phosphorothioates and phosphorodithioates were investigated. The compounds were evaluated for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition potency through IC_{50} determination. The selectivities of the synthesized compounds toward both enzymes were determined and compared in terms of their molecular structures.

1. Introduction

Organophosphorus compounds have found a wide range of applications in the areas of industrial, agricultural, and medicinal chemistry, owing to their biological and physical properties as well as their utility as synthetic intermediates [1,2]. Among organophosphorus compounds, phosphorothioates and dithioates are important class of compounds that exhibit a variety of interesting and useful properties in the area of medicinal chemistry owing to their biological and physical properties, as well as their utility as synthetic intermediates [3]. One of the useful properties of phosphorothioate compounds is their relatively low stability and rapid metabolic breakdown in plants, animals, and soil microorganisms, with the consequent formation of products that are environmentally safe for human beings and domestic animals [4]. Phosphorothioates have been prepared as pesticides and biologically active phosphoric mono- and di-esters [5,6].

Phosphorothioate Phosphorodithioate

Acetylcholinesterase (AChE) is the most widely used target enzyme in studies with the purpose to synthesize new and more effective therapeutic agents to treat patients with diseases such as Alzheimer's disease or myasthenia gravis [7–9]. Also, acetylcholinesterase is used widely as a target in the studies of compounds with insecticidal activities, as all commercially available organophosphate compounds are believed to exert their effects through inhibition of this enzyme [10]. In recent years a number of phosphorothioates have been introduced as potential chemotherapeutic agents [11,12] and inhibitors of different enzymes [13]. Despite their wide range of pharmacological activities, the synthesis and studies of anticholinesterase activities of novel phosphorothioate and phosphorodithioates have received little attention [14]. Butyrylcholinesterase (BChE) is a secondary target in organophosphorus compound poisoning and is of interest to monitor their exposure [15,16].

Recently a novel synthetic method was reported for the preparation of phosphorothioates and phosphorodithioates [17–19]. In the continuation of that study we decided to investigate to what extent the inhibitory effect of the synthesized phosphorothioates toward electric eel AChE would be affected upon the inclusion of a heteroatom in the aromatic *R* groups. Afterwards we compared the inhibitory effect of these phosphorodithioates with their phosphorothioate counterparts. Finally we investigated whether they would show any differential inhibitory effect on BChE.

2. Materials and methods

2.1. General

Chemicals were either prepared in our laboratories or purchased from Merck, Fluka, and Aldrich Chemical Companies. All yields refer to isolated products. NMR spectra were taken by a 250 Brucker with the chemical shifts being reported as δ ppm and couplings expressed in Hertz. Silica gel column chromatography was carried out with Silica gel 100 (Merck No. 10184). UV spectra were determined on a Varian Cary 100 instrument.

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Acetylcholinesterase from electric eel and butyrylcholinesterase (BChE) from horse serum, actetylcholine chloride, and gelatin were from Sigma and were used without further treatment. Acetylcholinesterase and butyrylcholinesterase activities were measured using Ellman method [20].

2.2. Synthesis of ammonium O,O′-diethylthiophosphate using of a mixture of ammonium hydrogen carbonate, sulfur, and diethylphosphite [17]

Ammonium hydrogen carbonate (0.1 mol, 7.9 g) was added to a mixture of sulfur (0.1 mol, 3.2 g) and diethylphosphite (0.1 mol, 13.8 g) in EtOAc/Et_2O (300 mL, 1:1). Reaction mixture was stirred for 24 h at room temperature. Evaporation of the solvent under reduced pressure gave the pure ammonium *O,O′*-diethylthiophosphate as solid in a quantitative yield that could be recrystallized in ethanol.

2.3. Synthesis of ammonium O,O′-diethyldithiophosphate using of a mixture of ammonium hydrogen carbonate, phosphorus pentoxide, and ethanol [18]

Ammonium hydrogen carbonate (0.1 mol, 7.9 g) was added to a mixture of phosphorus pentoxide (50 mmol, 11.1 g) in EtOH (100 mL) at 60 °C. Reaction mixture was stirred for 1 h at 60 °C. Evaporation of the solvent under reduced pressure gave the pure ammonium *O,O′*-diethyldithiophosphate as solid in quantitative yield that could be recrystallized in ethanol.

2.4. General procedure for the synthesis of phosphorothioates (**2**) [17]

Ammonium *O,O′*-diethyl phosphorothioate (5 mmol, 0.935 g) was dissolved in acetonitrile (10 mL). Alkyl halide (5 mmol) was added to reaction mixture and stirred for 24 h at reflux. Evaporation of solvent and washing with sodium hydrogen carbonate (50 × 3 mL) gave crude product *S*-alkyl *O,O′*-diethyl phosphorothioate. Chromatography through a plug of silica gel with EtOAc/*n*-hexane (1:1) and evaporation of the solvent under reduced pressure gave the pure products as oils in 34–80% yields. All products gave satisfactory spectral data in accord with the assigned structures.

2.5. General procedure for the synthesis of phosphorodithioates (**3**) [18]

Solvent-free reaction method, which is operationally simple, was used to synthesize phosphorodithioates. Ammonium *O,O′*-diethyl phosphorodithioate (5 mmol, 0.935 g) was added to alumina (Al_2O_3, acidic, 2.5 g). Alkyl halide (5 mmol) was added to reaction mixture and grinded for 1–2 h. The mixture was washed with chloroform (100 mL) and neutralized with sodium hydrogen carbonate (50 × 3 mL). Solvent was evaporated to give crude product alkyl *O,O′*-diethyl phosphorodithioate. Chromatography through a plug of silica gel with EtOAc/*n*-hexane (1:9) and evaporation of the solvent under reduced pressure gave the pure products as oils in 70–85% yields. All products gave satisfactory spectral data in accord with the assigned structures.

2.5.1. S-pyridin-4-ylmethyl-O,O′-diethyl phosphorothioate (**2a**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.30 (6H, t, J = 7.1 Hz), 3.99–4.13 (6H, m), 7.34 (2H, d, J = 5.9 Hz), 8.59 (2H, d, J = 5.9 Hz); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 26.21; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 16.3 (d, J_{pc} = 7.29 Hz), 33.4 (d, J_{pc} = 3.8 Hz), 64.2 (d, J_{pc} = 6.0 Hz), 124.2, 147.3 (d, J_{pc} = 4.3 Hz), 150.3. Anal. Calcd for $C_{10}H_{16}NO_3PS$: C, 45.97; H, 6.18; N, 5.36. Found: C, 45.83; H, 6.10; N, 5.25.

2.5.2. S-pyridin-2-ylmethyl-O,O′-diethyl phosphorothioate (**2b**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.17 (6H, t, J = 7.0 Hz), 3.92–4.07 (6H, m), 7.10 (1H, m), 7.30 (1H, m), 7.55 (1H, m), 8.45 (1H, m); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 26.61; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.8 (d, J_{pc} = 7.4 Hz), 36.4 (d, J_{pc} = 3.7 Hz), 63.5 (d, J_{pc} = 5.8 Hz), 122.3 (d, J_{pc} = 8.6 Hz), 123.9, 136.6 (d, J_{pc} = 14.4 Hz), 149.5 (d, J_{pc} = 5.8 Hz), 156.9 (d, J_{pc} = 4.8 Hz). Anal. Calcd for $C_{10}H_{16}NO_3PS$: C, 45.97; H, 6.18; N, 5.36. Found: C, 45.78; H, 6.20; N, 5.23.

2.5.3. S-quinolin-2-ylmethyl-O,O′-diethyl phosphorothioate (**2c**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.26 (6H, t, J = 7.0 Hz), 3.98–4.22 (4H, m), 4.33 (2H, d, J = 14.5 Hz), 7.48–7.56 (2H, m), 7.66–7.80 (2H, m), 8.02–8.14 (2H, m); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 26.68; ^{13}C NMR ($CDCl_3$ – 26.9 MHz): δ = 15.9 (d, J_{pc} = 7.4 Hz), 37.0 (d, J_{pc} = 3.8 Hz), 63.7 (d, J_{pc} = 5.9 Hz), 121.1, 126.6, 127.1, 127.5, 128.9, 129.8, 137.0, 147.6, 157.3 (d, J_{pc} = 5.1 Hz). Anal. Calcd for $C_{14}H_{18}NO_3PS$: C, 54.00; H, 5.83; N, 4.50. Found: C, 53.86; H, 5.75; N, 4.38.

2.5.4. S-[6-(diethoxy-thiophosphorylsulfanylmethyl)-pyridin-2-ylmethyl]-O,O′-diethyl phosphorothioate (**2d**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.30 (12H, t, J = 7.1 Hz), 4.05–4.17 (12H, m), 7.32 (2H, d, J = 7.7 Hz), 7.64 (1H, t, J = 7.7 Hz); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 27.18; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 16.3 (d, J_{pc} = 7.3 Hz), 36.7 (d, J_{pc} = 1.9 Hz), 64.0 (d, J_{pc} = 6.0 Hz), 122.3, 137.9, 157.5 (d, J_{pc} = 5.2 Hz). Anal. Calcd for $C_{15}H_{27}NO_6P_2S_2$: C, 40.63; H, 6.14; N, 3.16. Found: C, 40.90; H, 6.33; N, 3.29.

2.5.5. S-[2-(1,3-dioxo-1,3-dihidro-isoindol-2-yl)-ethyl]-O,O′-diethyl phosphorothioate (**2e**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.21 (6H, t, J = 7.0 Hz), 3.03 (2H, m), 3.85 (2H, m), 4.02 (4H, m), 7.59–7.72 (4H, m); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 26.62; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.9 (d, J_{pc} = 7.2 Hz), 28.6 (d, J_{pc} = 4.0 Hz), 38.1 (d, J_{pc} = 4.3 Hz), 63.7 (d, J_{pc} = 6.1 Hz), 123.2, 131.7, 134.0, 167.7. Anal. Calcd for $C_{14}H_{18}NO_5PS$: C, 48.97; H, 5.29; N, 4.08. Found: C, 48.81; H, 5.12; N, 4.19.

2.5.6. S-pyridin-4-ylmethyl-O,O′-diethyl phosphorodithioate (**3a**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.30 (6H, t, J = 7.1 Hz), 3.98–4.19 (6H, m), 7.50 (2H, d, J = 5.7 Hz), 8.60 (2H, d, J = 5.7 Hz); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 92.87; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.7 (d, J_{pc} = 8.2 Hz), 36.5 (d, J_{pc} = 3.1 Hz), 64.4 (d, J_{pc} = 6.3 Hz), 124.9, 141.5, 147.2. Anal. Calcd for $C_{10}H_{16}NO_2PS_2$: C, 43.31; H, 5.82; N, 5.05. Found: C, 43.15; H, 5.68; N, 5.15.

2.5.7. S-pyridin-2-ylmethyl-O,O′-diethyl phosphorodithioate (**3b**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.30 (6H, t, J = 7.25 Hz), 3.95–4.35 (6H, m), 7.30 (1H, m), 7.43 (1H, m), 7.69 (1H, m), 8.57 (1H, m); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 93.44; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.7 (d, J_{pc} = 8.2 Hz), 38.9 (d, J_{pc} = 3.8 Hz), 64.1 (d, J_{pc} = 6.3 Hz), 122.5, 123.6, 137.2, 149.0, 156. 7 (d, J_{pc} = 5.0 Hz). Anal. Calcd for $C_{10}H_{16}NO_2PS_2$: C, 43.31; H, 5.82; N, 5.05. Found: C, 43.25; H, 5.62; N, 5.10.

2.5.8. S-quinolin-2-ylmethyl-O,O′-diethyl phosphorodithioate (**3c**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.28 (6H, t, J = 7.2 Hz), 4.00–4.20 (4H, m), 4.45 (2H, d, J = 16.0 Hz), 7.54–7.62 (2H, m), 7.72–7.85 (2H, m), 8.13–8.21 (2H, m); ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 93.32; ^{13}C NMR ($CDCl_3$ – TMS – 62.9 MHz): δ = 15.8 (d, J_{pc} = 8.2 Hz), 38.1, 64.3 (d, J_{pc} = 6.3 Hz), 121.5, 129.4, 130.9, 134.2, 144.3, 156.9 (d, J_{pc} = 5.03 Hz). Anal. Calcd for $C_{14}H_{18}NO_2PS_2$: C, 51.37; H, 5.55; N, 4.28. Found: C, 51.20; H, 5.73; N, 4.35.

2.5.9. S-[6-(diethoxy-thiophosphorylsulfanylmethyl)-pyridin-2-ylmethyl]-O,O′-diethyl phosphorodithioate (**3d**)

^{1}H NMR ($CDCl_3$ – 250 MHz): δ = 1.30 (12H, t, J = 7.1 Hz), 4.00–4.30 (12H, m), 7.29 (2H, d, J = 7.7 Hz), 7.61 (1H, t, J = 7.72 Hz), ^{31}P

NMR ($CDCl_3$–H_3PO_4): δ = 93.48; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.8 (d, J_{pc} = 8.2 Hz), 39.0 (d, J_{pc} = 3.8 Hz), 64.1 (d, J_{pc} = 5.7 HZ), 121.9, 137.3, 156.9 (d, J_{pc} = 5.7 Hz). Anal. Calcd for $C_{15}H_{27}NO_4P_2S_4$: C, 37.89; H, 5.73; N, 2.95. Found: C, 37.75; H, 5.66; N, 3.10.

*2.5.10. S-[2-(1,3-dioxo-1,3-dihidro-isoindol-2-yl)-ethyl]-O,O′-diethyl phosphorodithioate (**3e**)*

^1H NMR ($CDCl_3$ – 250 MHz): δ = 1.35 (6H, t, J = 6.50 Hz), 3.13–3.26 (2H, m), 3.97 (2H, m), 4.1–4.3 (4H, m), 7.69–7.77 (2H, m), 7.82–7.89 (2H, m), ^{31}P NMR ($CDCl_3$–H_3PO_4): δ = 93.51; ^{13}C NMR ($CDCl_3$ – 62.9 MHz): δ = 15.8 (d, J_{pc} = 8.4 Hz), 31.2 (d, J_{pc} = 3.9 Hz), 38.0 (d, J_{pc} = 4.4 Hz), 64.2 (d, J_{pc} = 6.0 Hz), 123.45 (d, J_{pc} = 7.5 Hz), 131.9 (d, J_{pc} = 6.3 Hz), 134.2 (d, J_{pc} = 5.4 Hz), 167.9. Anal. Calcd for $C_{14}H_{18}NO_4PS_2$: C, 46.79; H, 5.05; N, 3.90. Found: C, 46.63; H, 5.16; N, 3.89.

3. Results and discussion

Novel phosphorothioates and phosphorodithioates were prepared by the reaction of alkyl halides with diethyl ammonium phosphorothioates and phophorodithioates (Scheme 1 and Table 1) [17–19]. Newly synthesized compounds were assayed for AChE (*Electrophorus electricus*) and butyrylcholinesterase (BChE) (Horse serum) inhibition potency by the Ellman method [17]. The results are summarized in Table 1.

As it can be seen in Table 1, phosphorothioates have generally behaved as more potent inhibitors of both AChE and BChE than phosphorodithioates. For example, in case of the most potent inhibitors, compounds **2e** and **3j**, the IC_{50} values were 1.18 μM and 4.05 μM, respectively. As another example, the IC_{50} values of compounds **2d** and **3i** (the least potent inhibitors) were 91.65 μM and 352.1 μM, respectively. It seems that the presence of P═O instead of P═S in the phosphonate moieties of thioate inhibitors, and their stronger interaction with the serine residues in the esteratic locus of the active sites in both enzymes has been the reason for their relatively more potency [21].

A comparison can also be made between the synthesized phosphorothioates and phosphorodithioates with respect to their selectivity toward the enzymes. Phosphorothioates **2a**–**2c** showed more selectivity to BChE (Table 1) (with selectivities 15.3, 14.4, and 15.7, respectively). Compounds **2d** and **2e** were also selective (4.0 and 5.4, respectively) toward BChE, but not as much as compounds **2a**–**2c**.

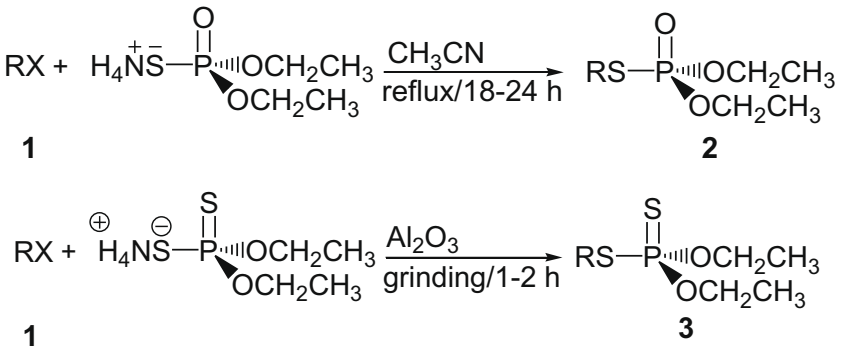
All phosphorodithioates (except compound **3i**), were almost equally potent toward both enzymes (selectivities were 1.2, 1.0, 1.3, and 1.2 for compounds **3f, 3g, 3h**, and **3j**, respectively).

In order to explain the general selectivity of phosphorothioates toward BChE and almost equal affinity of phosphorodithioates to both enzymes (except compound **3i**) we should consider the ligand binding sites in both enzymes, i.e., active site and peripheral anionic site (PAS). In terms of the active sites, it is known that the active site volume in BChE is larger than AChE [22–24] and hence can accommodate ligands with larger molecular structures. In PAS, and also in the distance from PAS to the active site (i.e., along the gorge structure), there are a number of hydrophobic amino acids, which in AChE they are largely aromatic and in BChE, they are largely aliphatic [25–27]. The larger active site volume in BChE, probably cannot provide an explanation for the general selectivity of phosphorothioates toward BChE, since there is no considerable difference in molecular volume among synthesized compounds, excluding compounds **2d** and **3i**. Since the interaction of ligands with PAS affects their interactions with the active sites of both enzymes [28–34], we may hypothesize that the reversible π–π interactions between phosphorothioates (especially **2a**–**2c**) and the aromatic residues in PAS and gorge structure of AChE has made their interactions with the active site residues in AChE less favorable and has inhibited the irreversible step of the reaction at the active center, which has resulted in higher selectivity for BChE (with lower IC_{50}'s).

In case of phosphorodithioates, such a selectivity was not observed (except for **3i**), although they also contained aromatic groups in their structures. It seems that the higher potency of phosphorothioates has made the different interactions between them and each of the enzymes to become more apparent. This conclusion needs further investigation.

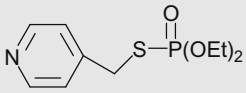
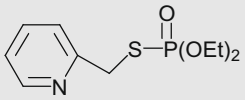
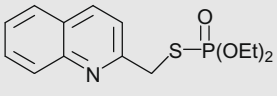
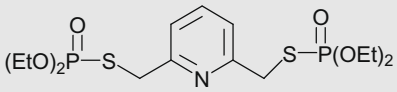
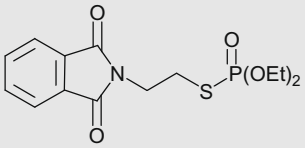
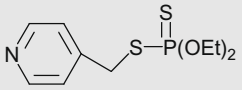
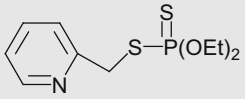
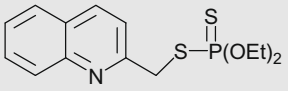
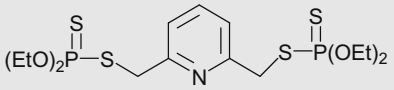
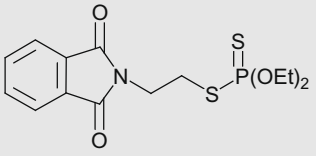
As shown in Table 1, the most potent inhibitors of both AChE and BChE were compounds **2e** and **3j**, respectively. The structural difference between these two compounds and the other ones is that in these two compounds there exist two methylene groups between the phosphonic and aromatic moieties of the compounds, whereas in the other compounds there is only one methylene group at the corresponding positions. Since there exists a hydrophobic region between the esteratic and anionic loci of cholinesterases [35–37], it can be postulated that the presence of these two methylene groups in compounds **2e** and **3j** has made their positioning in the hydrophobic region of the active site of both enzymes, i.e., cation–π interactions with Trp84 (AChE from *Torpedo californica* numbering), and its corresponding residue in BChE, Trp82 (Human BChE numbering), more optimized, and consequently have made their interactions with the enzymes stronger than the other compounds. This mechanism might explain their greatest inhibitory potency toward both of the enzymes.

Compounds **2d** and **3i** were the weakest inhibitors of both enzymes. According to their IC_{50}'s, they also showed selectivity toward BChE, which could be explained by the differences between the sizes of active sites and gorge structures in AChE and BChE. It has been shown that amino acid residues in the acyl pocket [37] and also in the peripheral anionic site of BChE [23], around the



Scheme 1. Synthesis of phosphorothioates and phosphorodithioates.

Table 1
Synthesis of novel phosphorothioate and phosphorodithioates and their anticholinesterase and butyrylcholinesterase activities.

Entry	Product	Time (h)	Yield[a] %	AChE IC_{50} (μM)	BChE IC_{50} (μM)	Selectivity to BChE[b]
a		24	70	19.60 ± 0.01	1.280 ± 0.01	15.3
b		24	34	10.63 ± 0.01	0.74 ± 0.008	14.4
c		18	53	7.390 ± 0.002	0.47 ± 0.00	15.7
d		24	80	91.65 ± 0.01	22.86 ± 0.013	4.0
e		24	65	1.180 ± 0.005	0.22 ± 0.00	5.4
f		1	80	23.95 ± 0.03	17.82 ± 0.001	1.3
g		1	85	16.87 ± 0.01	16.86 ± 0.02	1.0
h		1	80	15.42 ± 0.00	12.01 ± 0.002	1.3
i		2	83	352.1 ± 5.43	56.84 ± 0.002	6.2
j		2	70	4.05 ± 0.002	3.37 ± 0.002	1.2

[a] Yields refer to the isolated pure products after column chromatography.
[b] The selectivity for BChE is defined as IC_{50} (AChE)/IC_{50} (BChE).

periphery of the gorge, are such that provide larger opening and also larger active site region in BChE in comparison with AChE [21], as pointed out before. Their relative weakness in inhibitory potency is explainable by two facts: presence of two thionophosphonate moieties in their structures, and their molecular sizes. The existence of two thionophosphonate moieties, and the fact that only one of them could interact with the serine residues in the active sites of enzymes at the same time, makes the interaction of the second thiono or dithiono groups with active site residues more problematic by exerting a steric hinderance effect, which have made the interactions of the compounds **2d** and **3i** with other residues in the active sites less favorable.

According to the data in Table 1, the following order can be seen in the inhibitory potency, against both of the enzymes: **2c** > **2b** > **2a** and **3h** > **3g** > **3f**. Compounds **2c** and **3h** with their phenyl structure attached to the heterocyclic aromatic ring provide better situation for the nitrogen to be more positive (considering the electron withdrawing property of the aromatic ring) and hence stronger cation–π interactions with the catalytic anionic sites of the enzymes.

The structural difference between compounds **2a** and **2b** and also between their dithioate counterparts (**3f** and **3g**) is only in the position of nitrogen in the heterocyclic aromatic ring (*para* and *ortho* positions). Nitrogens in *ortho* position (compounds **2b** and **3g**), could interact better with the anionic sites of the enzymes because they would act as better chelators. In compounds **2a** and **3f** there would be no chelation process because of the *para* position of nitrogen in the aromatic rings and hence poorer interactions between them and the catalytic sites of the enzymes.

In conclusion this work showed that although the synthesized phosphorothioates were more potent inhibitors of both AChE and BChE, they behaved more selectively toward BChE. This selectivity was not observed in the synthesized phosphorodithioates, except compound **3i**, which was the bulkiest compound. Another observation was seemingly the importance of optimal positioning of compounds **2e** and **3j** in the active sites of both enzymes, presumably because of the presence of two methylene groups in their structures, and reflected in their IC_{50}'s, which were the lowest. The last, but not least, is that the data presented might also provide further evidence for the importance of PAS in the interactions between organophosphates and cholinesterases. As proposed earlier in the literature [28–34], the interactions between inhibitors and PAS, might inhibit the irreversible step in the active site through a number of mechanisms: by the consequent blocking of the entrance of the gorge, by exerting a conformational effect on active site and hence its interactions with inhibitor molecules or both.

According to the data presented above it seems that compounds **2e** and **3j** are good candidates for further optimizations studies because: (1) they showed acceptable potency toward AChE as the main target in insecticidal studies and (2) they also inhibit significantly BChE (although to different degrees) which is of importance considering the buffering effect of this enzyme in developing effective insecticides and also considering the poisoning effects of insecticides on mammals that could be reduced considerably if they can also bind effectively with BChE.

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References

[1] R.L. Hilderbrand, The Role of Phosphonates in Living Systems, CRC Press, Boca Raton, Fl, 1982.
[2] B. Kaboudin, Phosphorus Sulfur Silicon Relat. Elem. 177 (2002) 1749.
[3] C. Fest, K.-J. Schmidt, The Chemistry of Organophosphorus Pesticides, Springer-Verlag, Berlin–Heidelberg–New York, 1982.
[4] S.T. Crooke, C.F. Bennett, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 107.
[5] M.I. Elzagheid, K. Mattila, M. Oivanen, B.C.N.M. Jones, R. Cosstick, H. Lonnberg, Eur. J. Org. Chem. (2000) 1987.
[6] B. Kaboudin, S. Emadi, H. Norouzi, Phosphorus Sulfur Silicon Relat. Elem. 179 (2004) 585.
[7] A. Enz, R. Amstutz, H. Boddeke, G. Gmelin, J. Malanowski, Prog. Brain Res. 98 (1993) 431.
[8] C.B. Millard, C.A. Broomfield, J. Neurochem. 64 (1995) 1909.
[9] A. Lindner, B. Schalke, K.V. Toyka, J. Neurol. 244 (1997) 515.
[10] J.L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, Science 253 (1991) 872.
[11] E. Uhlman, A. Peyman, Chem. Rev. 90 (1990) 543.
[12] C.A. Stein, Y.C. Cheng, Science 261 (1993) 1004.
[13] M. Yoshido, T. Maeda, H. Sugiyama, Japanese Patent 1541 (C1.16 C 92), 1967;
M. Yoshido, T. Maeda, H. Sugiyama, Chem. Abstr. 66 (1967) 115455.
[14] M.I. Kabachnik, A.A. Abduvakhabov, I.I. Agabekova, A.P. Brestkin, Russ. Chem. Rev. 39 (1970) 485.
[15] J.E. Casida, G.B. Quistad, Chem. Res. Toxicol. 17 (2004) 983.
[16] F.M. Buratti, C. Leoni, E. Testai, J. Consum. Protect. Food Safety 2 (2007) 37.
[17] B. Kaboudin, F. Farjadian, Beilstein J. Org. Chem. 2 (2006) 4.
[18] B. Kaboudin, H. Norouzi, Tetrahedron Lett. 45 (2004) 1283.
[19] B. Kaboudin, Tetrahedron Lett. 43 (2002) 8713.
[20] G.L. Ellman, K.D. Countney, V. Andres, R.M. Featherstone, Biochem. Phamacol. 7 (1961) 88.
[21] D.M. Maxwell, K.M. Brecht, Chem. Res. Toxicol. 5 (1992) 66.
[22] D.C. Vellom, Z. Radić, Y. Li, N.A. Pickering, S. Camp, P. Taylor, Biochemistry 32 (1993) 12.
[23] P. Masson, M.T. Froment, C.F. Bartels, O. Lockridge, Eur. J. Biochem. 235 (1996) 36.
[24] Y. Nicolet, O. Lockridge, P. Masson, J.C. Fontecilla-Camps, F. Nachon, J. Biol. Chem. 278 (2003) 41141.
[25] D. Kaplan, A. Ordentlich, D. Barak, N. Ariel, C. Kronman, B. Velan, A. Shafferman, Biochemistry 40 (2001) 7433.
[26] M. Harel, J.L. Sussman, E. Krejci, S. Bon, P. Chanal, J. Massoulie, I. Silman, Proc. Natl. Acad. Sci. USA 89 (1992) 10827.
[27] M. Cyger, J.D. Schrag, J.L. Sussman, M. Harel, I. Silman, M.K. Gentry, B.P. Doctor, Protein Sci. 2 (1993) 366.
[28] A. Friboulet, F. Rieger, D. Goudou, G. Amitai, P. Taylor, Biochemistry 29 (1990) 914.
[29] T. Szegletes, W.D. Mallender, T.L. Rosenberry, Biochemistry 37 (1998) 4206.
[30] T. Szegletes, W.D. Mallender, P.J. Thomas, T.L. Rosenberry, Biochemistry 38 (1999) 122.
[31] W.D. Mallender, T. Szegletes, T.L. Rosenberry, J. Biol. Chem. 274 (1999) 8491.
[32] Y. Bourne, P. Taylor, Z. Radić, P. Marchot, EMBO J. 22 (2003) 1.
[33] D. Branduardi, F.L. Gervasio, A. Cavalli, M. Recanatini, M. Parrinello, J. Am. Chem. Soc. 127 (2005) 9147.
[34] J.P. Colletier, D. Fournier, H.M. Greenblatt, J. Stojan, J.L. Sussman, G. Zaccai, I. Silman, M. Weik, EMBO J. 25 (2006) 2746.
[35] D.M. Quinn, Chem. Rev. 87 (1987) 955.
[36] P. Taylor, J. Biol. Chem. 266 (1991) 4025.
[37] J.L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, Science 253 (1991) 872.