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Inhibitory Effects of O,O-Dialkyl S-Substituted Phosphorothioates on Acetylcholinesterase from the Moroccan and Asian Migratory Locusts

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A B S T R A C T

The inhibitory effects of the phosphorus-containing compounds BD-16, BD-17, and BD-18 (O,O-dialkyl S-(2,3-epoxypropyl) phosphorothioates) and BD-54 (O,O-diethyl S-(4-piperidinobut-2-ynyl) phosphorothioate), synthesized at the National University of Uzbekistan, were investigated using acetylcholinesterase (AChE) isolated from the heads of Moroccan locusts (Dociostaurus maroccanus Thunb.) and Asian migratory locusts (Locusta migratoria L.) collected in the Syrdarya Region. Enzyme activity was determined spectrophotometrically by the Ellman method at 412 nm, using inhibitor concentrations of 10^{-3}–10^{-6} M. Under control conditions, AChE activity in the Moroccan locust preparation (A_{30} = 1.535) was 4.2-fold higher than that in the Asian migratory locust preparation (A_{30} = 0.363). At 10^{-3} M, BD-16 inhibited Moroccan and Asian migratory locust AChE by 59.1% and 54.8%, respectively. At 10^{-6} M, its effect on the Moroccan locust enzyme was negligible (0.5%), whereas inhibition of the Asian migratory locust enzyme remained at 27.3%. BD-17 was the most active compound (69.8% inhibition at 10^{-3} M; $I_{50} \approx 1.8 \times 10^{-4}$ M), while BD-18 showed lower activity (47.5%). BD-54 inhibited AChE by 46.3% but had almost no effect on butyrylcholinesterase (≤5.8%). These findings suggest that alkyl-chain length and the nature of the S-substituent influence inhibitory activity and species-related selectivity.

Introduction

Locusts are among the most destructive agricultural pests in Central Asia, including Uzbekistan. During outbreaks, the Moroccan locust (Dociostaurus maroccanus) and the Asian migratory locust (Locusta migratoria) can damage extensive areas of pastureland and crops (1, 2). Chemical control, particularly the use of organophosphorus

insecticides, remains one of the principal methods for managing these pests.

The insecticidal action of organophosphorus compounds is associated with phosphorylation of the serine residue in the active site of acetylcholinesterase (AChE; EC 3.1.1.7) in the nervous system (3, 4). AChE hydrolyses acetylcholine at cholinergic synapses, terminating nerve impulse transmission; its inhibition leads to acetylcholine accumulation, paralysis, and ultimately insect death (5). The kinetic properties of insect AChE and its sensitivity to inhibitors can vary substantially among species, populations, and developmental stages. Such differences are among the mechanisms involved in the development of insecticide resistance (6, 7).

In our previous studies, the kinetic parameters of AChE isolated from the heads of Moroccan and oasis locusts occurring in the Syrdarya Region were determined using the Lineweaver–Burk method. The enzymes from the two species were shown to differ in their Michaelis constants (K_m) and maximum reaction rates (V_{max}) (8). Butyrylcholinesterase (BChE) activity was also compared in these species, revealing differences in substrate affinity and catalytic efficiency (9). These interspecific differences in enzyme kinetics may also be reflected in inhibitor sensitivity and could help identify compounds with species-selective effects.

The aim of this study was to determine the inhibitory effects of O,O-dialkyl S-substituted phosphorothioates (BD-16, BD-17, BD-18, and BD-54), synthesized at the National University of Uzbekistan, on AChE from Moroccan and Asian migratory locusts, and to assess the influence of alkyl-group length and S-substituent structure on inhibitory activity.

Materials and Methods

Inhibitors. Four phosphorus-containing compounds synthesized by Professor B. N. Boboyev of the National University of Uzbekistan were used (Fig. 1). BD-16, BD-17, and BD-18 form a homologous series of O,O-dialkyl S-(2,3-epoxypropyl) phosphorothioates and differ only in the alkyl group (R = C_2H_5, C_3H_7, or C_4H_9). In BD-54, the epoxypropyl group is replaced by a 4-piperidinobut-2-ynyl moiety; therefore, the molecule contains a protonatable tertiary nitrogen atom.

Insect collection and enzyme preparation. Moroccan and Asian migratory locusts collected in the Syrdarya Region were stored frozen at −20 °C. The heads were dissected and homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min at 0°C using a Dlab D1524R centrifuge (China). The enzyme-containing supernatant was used for activity measurements (8).

Enzyme activity assay. AChE activity was determined using the Ellman method (10). The assay is based on the reaction of thiocholine generated by substrate hydrolysis with 5,5′-dithiobis(2-nitrobenzoic acid), producing the yellow-coloured 5-thio-2-nitrobenzoate anion. The following reagents (Sigma-Aldrich) were used: (1) phosphate buffer, pH 7.4 (P3563), containing 0.05% Tween 20; (2) Ellman's reagent (D8130, >98%), prepared as a 2×10^{-3} M solution in phosphate buffer; and (3) acetylthiocholine iodide (A5626, >99%), prepared as a 1×10^{-3} M solution in distilled water. Butyrylthiocholine iodide was used as the substrate when assessing the effect of BD-54 on BChE (9).

A blank solution was first prepared from the reagents. Enzyme activity measured in the absence of inhibitor served as the control. For experimental samples, the enzyme was pre-incubated with inhibitor at concentrations of 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} M for 5 min, after which the substrate was added.

The absorbance (A) of the reaction mixture was measured at 412 nm at specified intervals over 30 min using a HACH DR3900 spectrophotometer (Germany).

Calculation of results. The percentage inhibition (I, %) was calculated from the absorbance measured at 30 min as follows:

$$I = ((A_k - A_i) / A_k) \times 100\%$$

Where A_k is the absorbance of the control sample and A_i is the absorbance of the sample containing the inhibitor. The concentration producing 50% inhibition (I_{50}) was estimated by linear interpolation of the inhibition-versus-$\log_{10}$ concentration relationship (11, 12).

Results and Discussion

A marked difference in enzyme activity was observed between the two species in the control samples. After 30 min, the absorbance for Moroccan locust AChE was 1.535, compared with 0.363 for Asian migratory locust AChE (Tables 1 and 2).

Table.1 Effect of BD-16 on AChE from the Moroccan locust (absorbance at 412 nm)

Time (min)	Control	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
5	0.218	0.276	0.331	0.359	0.375
10	0.616	0.417	0.556	0.652	0.697
15	0.923	0.511	0.734	0.912	0.974
20	1.195	0.566	0.878	1.138	1.219
25	1.398	0.604	0.986	1.316	1.413
30	1.535	0.628	1.061	1.482	1.527

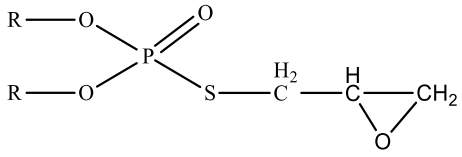
Table.2 Effect of BD-16 on AChE from the Asian migratory locust (absorbance at 412 nm)

Time (min)	Control	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
5	0.181	0.129	0.130	0.135	0.138
10	0.250	0.151	0.157	0.163	0.171
15	0.281	0.153	0.174	0.189	0.198
20	0.299	0.158	0.186	0.207	0.226
25	0.330	0.163	0.194	0.226	0.242
30	0.363	0.164	0.200	0.242	0.264

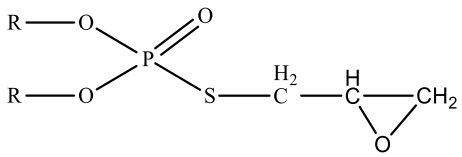
Table.3 Inhibition of locust AChE by the phosphorothioates (I, %, at 30 min)

Compound	Locust species	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	I_{50} (M)
BD-16	Moroccan	59.1	30.9	3.5	0.5	$\approx 4.7 \times 10^{-4}$
BD-16	Asian migratory	54.8	44.9	33.3	27.3	$\approx 3.3 \times 10^{-4}$
BD-17	Moroccan	69.8	43.1	39.3	34.3	$\approx 1.8 \times 10^{-4}$
BD-18	Moroccan	47.5	22.6	6.7	≈ 0	$> 10^{-3}$
BD-18	Asian migratory	30.9	20.7	12.1	3.2	$> 10^{-3}$
BD-54	Moroccan	46.3	43.1	20.5	11.4	$> 10^{-3}$

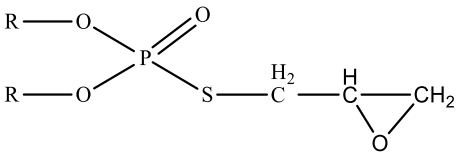
Figure.1 Structural formulae of the O,O-dialkyl S-substituted phosphorothioates investigated.



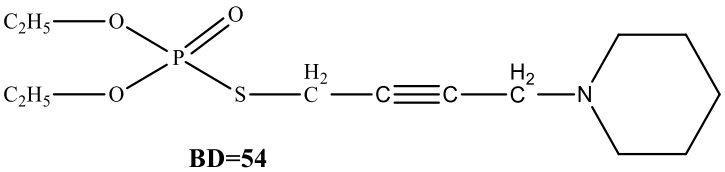
BD-16 ($R=C_2H_5$)



BD-17 ($R=C_3H_7$)

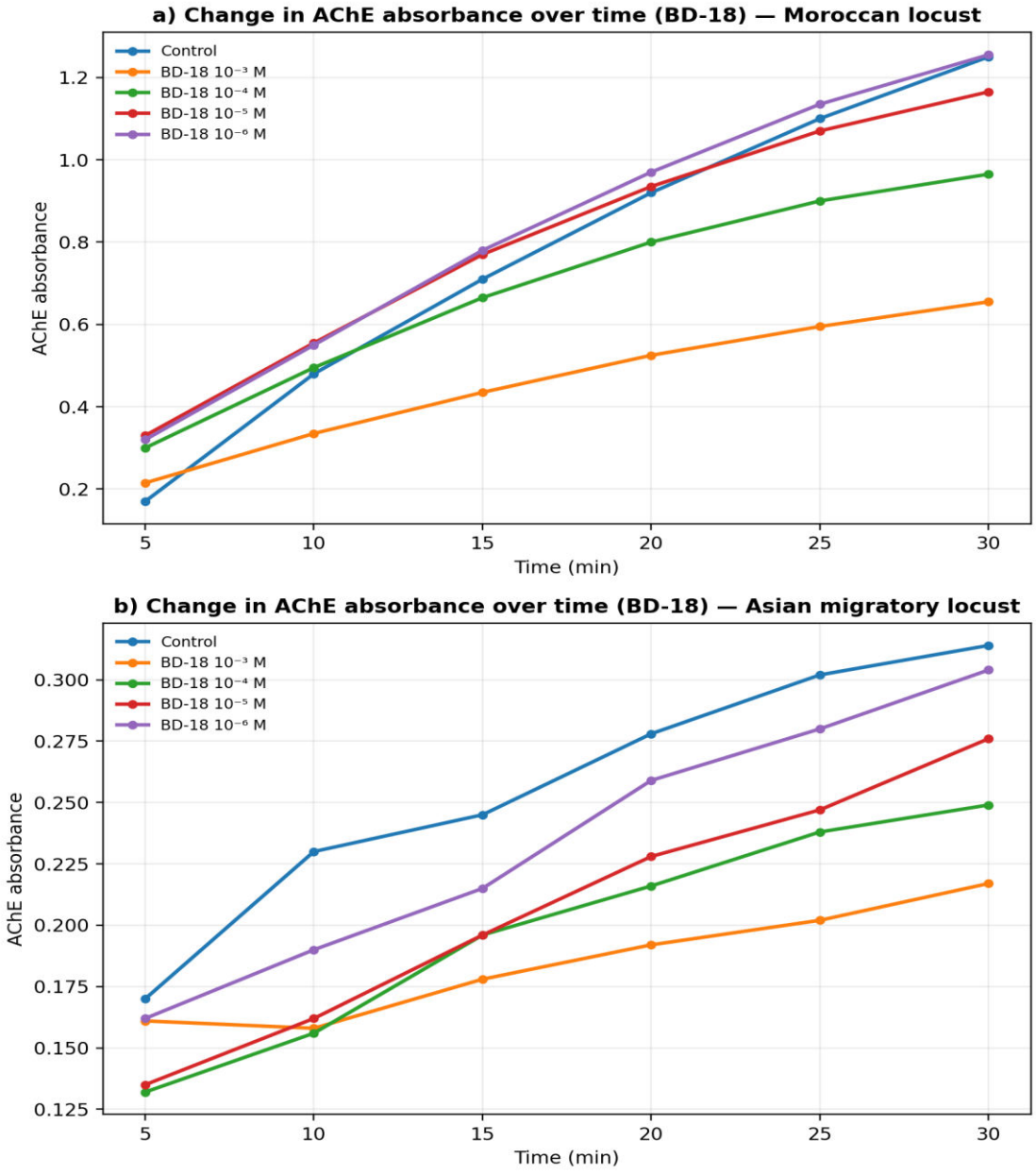


BD-18 ($R=C_4H_9$)



BD=54

Figure.2 Time-dependent changes in absorbance during AChE reactions in the presence of BD-18 in the Moroccan locust (a) and Asian migratory locust (b).



Thus, under identical conditions, the head extract of the Moroccan locust hydrolysed acetylthiocholine approximately 4.2 times more rapidly. This finding is consistent with the interspecific kinetic differences reported in our previous study (8).

The effect of BD-16 on Moroccan locust AChE was clearly concentration-dependent. At 10^{-3} and 10^{-4} M, the enzyme was inhibited by 59.1% and 30.9%, respectively, whereas the 10^{-5} and 10^{-6} M solutions produced little or no inhibition (3.5% and 0.5%). The higher baseline activity of the enzyme may make the effect of low inhibitor concentrations less apparent.

A different pattern was observed for the Asian migratory locust enzyme. Even at 10^{-6} M, BD-16 inhibited AChE by 27.3% (A_{30} = 0.264, compared with 0.363 in the control); at 10^{-5} M, inhibition was 33.3%. Although the

estimated I_{50} values for the two species were similar (Moroccan locust, approximately 4.7×10^{-4} M; Asian migratory locust, approximately 3.3×10^{-4} M), the inhibition curve for the Asian migratory locust enzyme was shallower. Thus, BD-16 may remain effective against the Asian migratory locust enzyme at relatively low concentrations.

The effect of BD-18 (R = C_4H_9) is shown in Fig. 2. It produced a substantial effect on Moroccan locust AChE only at 10^{-3} M (47.5% inhibition); inhibition decreased to 22.6% at 10^{-4} M and was negligible at 10^{-5}–10^{-6} M. For the Asian migratory locust enzyme, inhibition by BD-18 decreased steadily from 30.9% to 12.1% over the 10^{-3}–10^{-5} M concentration range.

The calculated inhibition values for all compounds are summarised in Table 3.

Effect of alkyl-chain length. For Moroccan locust AChE, activity increased as the alkyl group changed from ethyl (C_2H_5) to propyl (C_3H_7), with inhibition rising from 59.1% to 69.8%, and then decreased for the butyl derivative (C_4H_9; 47.5%). This apparently parabolic relationship has been described for organophosphorus inhibitors and may reflect a balance between increased lipophilicity with increasing alkyl-chain length and steric compatibility with the enzyme's acyl-binding pocket (11, 13). The propyl group may fit the active-site pocket most favourably, whereas the butyl group may introduce steric hindrance and reduce the rate of phosphorylation.

Because the BD-17 experiment was conducted in a separate series with its own control, this pattern should be confirmed in replicate experiments. Effect of the S-substituent. In BD-54, the epoxypropyl group is replaced by a piperidinobutynyl moiety. At physiological pH, the protonatable nitrogen atom may resemble the cationic head group of acetylcholine and interact with the enzyme's anionic (peripheral) site (5, 14). BD-54 therefore inhibited AChE by approximately 43–46% at 10^{-3}–10^{-4} M and, unlike BD-16, still produced 20.5% inhibition at 10^{-5} M. In contrast, BD-54 had almost no effect on BChE (≤5.8% at all tested concentrations), suggesting selectivity for AChE. Differences in substrate specificity between insect cholinesterases may provide a biochemical basis for this selectivity (9, 15).

Species-related selectivity. The results show that the same compound does not affect the two locust species identically. The higher AChE activity in the Moroccan locust preparation may reduce the apparent effect of inhibitors at low concentrations, whereas the Asian migratory locust enzyme remained sensitive even at 10^{-5}–10^{-6} M.

From an applied perspective, control of the Moroccan locust may require a higher dose or a more active homologue, such as BD-17. This interpretation requires validation in further biological assays.

1. AChE activity in head extracts of the Moroccan locust was 4.2 times higher than that in the Asian migratory locust (A_{30} = 1.535 and 0.363, respectively).

2. O,O-Dialkyl S-(2,3-epoxypropyl) phosphorothioates inhibited AChE from both species in a concentration-dependent manner. At 10^{-3} M, the activity ranking for the Moroccan locust was BD-17 (69.8%) > BD-16 (59.1%) > BD-18 (47.5%). A parabolic relationship with alkyl-group length was observed, with the propyl derivative showing the highest activity.

3. BD-16 inhibited Asian migratory locust AChE by 27.3% even at 10^{-6} M, whereas its effect on Moroccan locust AChE at this concentration was negligible (0.5%), indicating a species-related difference in sensitivity.

4. BD-54, which contains a piperidinobutynyl moiety, inhibited AChE by up to 46.3% but had almost no effect on BChE (≤5.8%), suggesting selectivity for AChE.

5. BD-17 and BD-16 are promising candidate compounds for further biological testing in the development of selective insecticidal agents targeting Moroccan and Asian migratory locusts.

Author Contributions

Ibragimova Shakhodat Khakimboyevna: Investigation, formal analysis, writing—original draft. Burkhev Farkhod Zabhievich: Validation, methodology, writing—reviewing. Babaev Bakhrom Nurillayevich:—Formal analysis, writing—review and editing. Kushiev Khabibjon Khojiboboevich: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the

current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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